LOWER PASSAIC RIVER RESTORATION PROJECT

LOWER PASSAIC RIVER STUDY AREA RI/FS

CAGED BIVALVE STUDY
ADDENDUM TO THE
QUALITY ASSURANCE PROJECT PLAN

SURFACE SEDIMENT CHEMICAL ANALYSES AND BENTHIC INVERTEBRATE TOXICITY AND BIOACCUMULATION TESTING

FINAL

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Revision Number: 0
Addendum Number 4

Prepared By:

Windward environmental LLC

200 West Mercer Street, Suite 401
Seattle, Washington 98119

623675

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Acronyms

COPEC	chemical of potential ecological concern
CPG	Cooperating Parties Group
CSO	combined sewer overflow
ERA	ecological risk assessment
HHRA	human health risk assessment
ID	Identification
LPRRP	Lower Passaic River Restoration Project
LPRSA	Lower Passaic River Study Area
MLLW	mean lower low water
NJDEP	New Jersey Department of Environmental Protection
NJDOT	New Jersey Department of Transportation
NOAA	National Oceanic and Atmospheric Administration
QAPP	quality assurance project plan
PA	Partner Agencies
PFD	problem formulation document
ppt	parts per thousand
RM	river mile
SOP	standard operating procedure
USACE	US Army Corps of Engineers
USEPA	US Environmental Protection Agency
USFWS	US Fish and Wildlife Service
Windward	Windward Environmental LLC

Introduction

This is an addendum to the *Lower Passaic River Restoration Project Quality Assurance Project Plan: Surface Sediment Chemical Analyses and Benthic Invertebrate Toxicity and Bioaccumulation Testing* (Windward 2009), hereafter referred to as the Benthic Quality Assurance Project Plan (QAPP). The Benthic QAPP, which was reviewed by the US Environmental Protection Agency (USEPA) and its Partner Agencies (PA)[1] and approved by USEPA on October 8, 2009, describes the sampling effort and data use objectives for tissue samples collected from the Lower Passaic River Study Area (LPRSA) to support the baseline ecological risk assessment (ERA) and the baseline human health risk assessment (HHRA) for the LPRSA. This addendum to the Benthic QAPP, hereafter referred to as Benthic QAPP Addendum No. 4, describes the *in situ* caged bivalve study that will be conducted at USEPA's direction and will be used to determine the potential for caged bivalves to be used as a long-term tool for monitoring chemicals in the water column prior to and following remediation in the LPRSA. In addition, per USEPA direction, chemical concentrations in tissue from caged bivalves will be used to assess the effects of LPRSA chemicals on bivalves and as a component in a food web model. Data to be collected will include the measurement (i.e., length) of each bivalve from each sampling location, survival numbers, and analytical tissue results from a single composite tissue sample from each location at the end of the exposure period. Additional data to be collected include analytical tissue results from each species pre-deployment (T_0 sample) to provide information on tissue concentrations at the start of the exposure period. Benthic QAPP Addendum No. 4 includes updates to worksheets relevant to the caged bivalve study; it does not include updates to those worksheets or attachments that are not relevant to this sampling event. Applicable and updated worksheets included in this addendum are presented below:

- Worksheet No. 1 contains the title and approval pages for the addendum.
- Worksheet No. 3 provides the distribution list.
- Worksheet No. 9 provides a record of relevant communication with USEPA/PA pertaining to the caged bivalve study.
- Worksheet No. 10 describes the specific problem defined for the caged bivalve study.
- Worksheet No. 11 provides a summary of project tasks.
- Worksheet No. 12 provides the measurement performance criteria table for polycyclic aromatic hydrocarbons (PAHs) (including alkylated PAHs).[2]
- Worksheet No 13 provides a summary of secondary data criteria and limitations.
- Worksheet No. 15 provides the data quality levels and analytical methods for PAHs (including alkylated PAHs).

[1] The Partner Agencies include the US Army Corps of Engineers (USACE), New Jersey Department of Environmental Protection (NJDEP), New Jersey Department of Transportation (NJDOT), National Oceanic and Atmospheric Administration (NOAA), and the US Fish and Wildlife Service (USFWS).

[2] Worksheets 12, 15, 24, and 28 are included in this QAPP addendum to provide information specific to the analysis of PAHs (including alkylated PAHs). Information on all other analytes is provided in the Benthic QAPP (Windward 2009).

- Worksheet No. 16 provides the schedule and timeline.
- Worksheet No. 18 provides a list of proposed sampling locations.
- Worksheet No. 21 provides the standard operating procedure (SOP) references table.
- Worksheet No. 23 provides the analytical and biological SOP references table.
- Worksheet No. 24 provides the analytical instrument calibration table for PAHs (including alkylated PAHs).
- Worksheet No. 27 provides the sample custody requirements, specifically the sample identification procedures.
- Worksheet No. 28 provides the quality control samples table for PAHs (including alkylated PAHs).
- Worksheet No. 29 provides a summary of project documents and records.
- Attachment U is an SOP for the deployment, monitoring, and processing procedures for the caged bivalve study.
- Attachment V provides the LPRSA Caged Bivalve Study Data Form.
- Attachment W is an SOP for tissue preparation and homogenization
- Attachment X is an SOP for the analysis of PAHs
- Attachment Y is an SOP for alumina column cleanup of organic extracts
- Attachment Z is the Caged Bivalve Pilot Study Results Memorandum

QAPP Worksheet No. 1. Title and Approval Page

Addendum to the *Quality Assurance Project Plan for Surface Sediment Chemical Analyses and Benthic Invertebrate Toxicity and Bioaccumulation Testing*

Document Title

Windward Environmental LLC (Windward)

Lead Investigative Organization

Joanna Florer, Windward

Preparer's Name and Organizational Affiliation

200 West Mercer St., Suite 401, Seattle, WA 98119, 206.378.1364, joannaf@windwardenv.com

Preparer's Address, Telephone Number, and E-mail Address

32/02/11

Preparation Date (mm/dd/yy)

Investigative Organization's Project Manager:

Signature

Lisa Saban, Windward, Date

Printed Name/Organization/Date

Investigative Organization's Task QA/QC Manager:

Signature

Tad Deshler, Windward, Date

Printed Name/Organization/Date

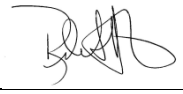
Project Coordinators:

Signature

Bill Potter, de maximis, inc., Date

Printed Name/Organization/Date

QAPP Worksheet No. 1. Title and Approval Page



Signature

Robert Law, de maximis, inc., Date

Printed Name/Organization/Date

Approval Signatures:

USEPA Project Manager

Approval Authority Signature

Stephanie Vaughn, USEPA, Date

Printed Name/Title/Date

USEPA Project QA Officer

Approval Authority Signature

William Sy, USEPA, Date

Printed Name/Title/Date

QAPP Worksheet No. 3. Distribution List

QAPP Recipients	Title	Organization	Telephone Number	E-mail Address
Lisa Saban	Investigative Organization Project Manager	Windward	206.812.5429	lisas@windwardenv.com
Mike Johns	Technical Advisory Team Member	Windward	206.812.5418	mikej@windwardenv.com
Tad Deshler	Investigative Organization Task QA/QC Manager	Windward	206.812.5406	tad@windwardenv.com
Susan McGroddy	Investigative Organization Project Chemist	Windward	206.812.5421	susanm@windwardenv.com
Kimberley Goffman	Investigative Organization Information Manager	Windward	206.812.5414	kimg@windwardenv.com
Jennifer Parker	Investigative Organization Data Validation Coordinator	Windward	206.812.5442	jenniferp@windwardenv.com
Thai Do	Field Coordinator/Site Safety and Health Officer	Windward	206.812.5407	thaid@windwardenv.com
Angelita Rodriquez	Field Coordinator/Site Safety and Health Officer (alternate)	Windward	512.436.8645	angelitar@windwardenv.com
Mike Yarnes	Field Personnel	Windward	206.812.5430	mikey@windwardenv.com
Sarah Fowler	Field Personnel	Windward	206.812.5440	sarahf@windwardenv.com
Bill Potter/Robert Law	Project Coordinators	de maximis, inc.	908.735.9315	otto@demaximis.com rlaw@demaximis.com
William Hyatt	Coordinating Counsel	K&L Gates	973.848.4045	william.hyatt@klgates.com
Jeff Clemens	Boat Operator	Aqua Survey, Inc.	908.347.3927	clemens@aquasurvey.com
Polly Newbold	CPG QA Coordinator	de maximis Data Management	908.479.1975	pnewbold@ddmsinc.com

QAPP Worksheet No. 3. Distribution List

QAPP Recipients	Title	Organization	Telephone Number	E-mail Address
		Solutions, Inc.		
Stella Cuenco	Third-Party Independent Validator	Laboratory Data Consultants	760.634.0437	scuenco@lab-data.com
Ellen Collins	Laboratory Project Manager	Alpha Analytical	508.317.4705	ecollins@alphalab.com
Kimberly Mace	Laboratory Project Manager	Analytical Perspectives	910.794.1613, ext. 102	kmace@ultratrace.com
Misty Kennard-Mayer	Laboratory Project Manager	Brooks Rand Labs	206.753.6125	Misty@brooksrand.com
Lynda Huckestein	Laboratory Project Manager	Columbia Analytical Services, Inc.	360.577.7222	LHuckestein@caslab.com
Mike Challis	Laboratory Project Manager	Maxxam Analytics	800.563.6266, ext. 5790	mike.challis@maxxamanalytics.com
Stephanie Vaughn	USEPA Project Manager	USEPA Region 2	212.637.3914	vaughn.stephanie@epamail.epa.gov
Chuck Nace	USEPA Risk Assessor	USEPA Region 2	212.637.4164	nace.charles@epa.gov
Lisa Baron	Project Manager	USACE	917.790.8306	Lisa.A.Baron@usace.army.mil
Janine MacGregor	Project Coordinator	NJDEP	609.633.0784	Janine.MacGregor@dep.state.nj.us
Timothy Kubiak	Assistant Supervisor of Environmental Contaminants	USFWS	609.646.9310, ext. 26	tim_kubiak@fws.gov
Reyhan Mehran	Coastal Resource Coordinator	NOAA	212.637.3257	reyhan.mehran@noaa.gov

QAPP Worksheet No. 9. Project Scoping Session Participants Sheet

Project Name:	Lower Passaic River Restoration Project (LPRRP) ERA and HHRA
Site Name:	LPRSA
Projected Date(s) of Sampling:	March 2011
Site Location:	LPRSA
Project Manager:	Bill Potter/Robert Law, de maximis, inc.
Date of Session:	June 25, 2009
Purpose of Session:	Conference call between USEPA and the Cooperating Parties Group (CPG) to discuss the problem formulation document (PFD) (Windward and AECOM 2009)

Participants: USEPA, de maximis, inc., AECOM, Windward

Name	Affiliation	Phone No.	E-mail Address
Stephanie Vaughn	USEPA	212.637.3914	vaughn.stephanie@epa.gov
Chuck Nace	USEPA	212.637.4164	nace.charles@epa.gov
Alice Yeh	USEPA	212.637.4427	yeh.alice@epa.gov
Robert Law	de maximis, inc.	908.735.9315	rlaw@demaximis.com
Betsy Ruffle	AECOM	978.589.3071	betsy.ruffle@aecom.com
Lisa Saban	Windward Environmental	206.812.5429	lisas@windwardenv.com

Conference call to discuss the PFD (Windward and AECOM 2009), which included a discussion of a caged bivalve study

Purpose/Decisions:	A conference call between USEPA and CPG to discuss USEPA's comments on the draft PFD was held on June 25, 2009. During the call, USEPA provided the following information regarding their position on conducting a caged bivalve study in the LPRSA: • USEPA directed that the PFD include a reference to a caged bivalve study and that a caged bivalve study be conducted as part of the LPRSA field effort. • USEPA said they would provide CPG with the methods for the study, which would include methods for conducting caged bivalve studies in both the estuarine and freshwater portions of the LPRSA. • USEPA agreed that that the field work for the caged bivalve study would be implemented as a stand-alone investigation to be started after the 2009 field sampling events.

QAPP Worksheet No. 9. Project Scoping Session Participants Sheet

Project Name:	Lower Passaic River Restoration Project (LPRRP) ERA and HHRA
Site Name:	LPRSA
Projected Date(s) of Sampling:	March 2011
Site Location:	LPRSA
Project Manager:	Bill Potter/Robert Law, de maximis, inc.
Date of Session:	April 25, 2010
Purpose of Session:	Conference call between USEPA and the Cooperating Parties Group (CPG) to discuss the proposed caged bivalve study

Participants: USEPA, de maximis, inc., ARCADIS, MAB Consulting, Windward

Name	Affiliation	Phone No.	E-mail Address
Stephanie Vaughn	USEPA	212.637.3914	vaughn.stephanie@epa.gov
Chuck Nace	USEPA	212.637.4164	nace.charles@epa.gov
Robert Law	de maximis, inc.	908.735.9315	rlaw@demaximis.com
Tim Iannuzzi	ARCADIS	410.295.1205	tim.iannuzzi@arcadis-us.com
Mike Barbara	MAB Consulting	973.543.5608	mab.consulting@verizon.net
Lisa Saban	Windward Environmental	206.812.5429	lisas@windwardenv.com
Mike Johns	Windward Environmental	206.812.5418	mikej@windwardenv.com

Conference call between USEPA/PA and CPG to discuss the proposed caged bivalve study	
Purpose/Decisions:	A conference call between USEPA/PA and CPG to discuss USEPA's proposed guidelines for the caged bivalve study was held on April 25, 2010, and included a discussion of the following concerns expressed by CPG: • CPG requested specific data quality objectives for the caged bivalve study from USEPA/PA. • USEPA/PA proposed the use of *Crassostrea virginica* (Eastern oyster) for the estuarine species. CPG preferred the use of *Geukensia demissa* (ribbed mussel) to be consistent with the Tierra Solutions caged bivalve study (Tierra Solutions 1999) and because *G. demissa* is tolerant of low salinity and may survive better than oysters in the upper portion (i.e., low salinity portion) of the estuarine zone. • USEPA/PA proposed that the caged bivalve study be conducted for 180 days. CPG recommended a 90-day deployment, which is consistent with methods provided by the American Society for Testing and Materials and other caged bivalve studies conducted at other sites. • USEPA/PA requested that sediment and water column chemical analyses be conducted on samples collected at the caged bivalve study locations. CPG countered that sufficient data are available from the 2009/2010 field efforts to provide this information. • USEPA/PA and CPG did not reach an agreement on the use of a reference area for the caged bivalve study.

QAPP Worksheet No. 9. Project Scoping Session Participants Sheet

Project Name:	LPRRP ERA and HHRA
Site Name:	LPRSA
Projected Date(s) of Sampling:	March 2011
Site Location:	LPRSA
Project Manager:	Bill Potter/Robert Law, de maximis, inc.
Date of Session:	A series of discussions between May and December 2010
Purpose of Session:	Following the April 25, 2010, conference call, USEPA and CPG held a series of discussions to determine the scope of the caged bivalve study

Participants: USEPA, de maximis, inc., Windward

Stephanie Vaughn	USEPA	212.637.3914	vaughn.stephanie@epa.gov
Chuck Nace	USEPA	212.637.4164	nace.charles@epa.gov
Robert Law	de maximis, inc.	908.735.9315	rlaw@demaximis.com
Lisa Saban	Windward Environmental	206.812.5429	lisas@windwardenv.com
Mike Johns	Windward Environmental	206.812.5418	mikej@windwardenv.com

USEPA directed that CPG conduct a caged bivalve study in the LPRSA and provided method guidelines for CPG to consider (e.g., species, number of locations, water quality measurements).

Purpose/Decisions:	A summary of some of the methods that USEPA and CPG agreed to include in the caged bivalve study are as follows: • Estuarine species will be determined following completion of a pilot study conducted to compare the survivability of the Eastern oyster, *Crassostrea virginica*, and the ribbed mussel, *Geukensia demissa*, at two locations in the estuarine portion of LPRSA (Windward 2010b). • Organisms will be acclimated to LPRSA conditions prior to deployment to ensure that there are no transport problems or mortality and to avoid temperature/salinity shock. • Sufficient numbers of organisms will be included per cage to meet the minimum tissue mass needed at the end of the 90-day exposure period plus additional individuals per location to sacrifice at mid-test check points throughout the study to assess general health. • Eight sampling locations will be selected in the LPRSA. • The caged bivalve study will be conducted for 90 days starting in March 2011. • Cages will be checked at two weeks intervals for the first month of deployment and then at approximately one-month intervals until test termination. • Water quality (e.g., salinity/conductivity, temperature) will be measured and recorded at deployment, at mid-test check points, and at termination. • The caged bivalve study will not include the use of a reference area, since the study is only being used to determine the potential for caged bivalves to be used as a long-term tool for monitoring chemicals in the water column prior to and following remediation in the LPRSA.

QAPP Worksheet No. 9. Project Scoping Session Participants Sheet

Project Name:	Lower Passaic River Restoration Project (LPRRP) ERA and HHRA
Site Name:	LPRSA
Projected Date(s) of Sampling:	March 2011
Site Location:	LPRSA
Project Manager:	Bill Potter/Robert Law, de maximis, inc.
Date of Session:	January 20, 2011
Purpose of Session:	Conference call between USEPA and the CPG to discuss results from the caged bivalve pilot study

Participants: USEPA, de maximis, inc., CDM, Windward

Name	Affiliation	Phone No.	E-mail Address
Stephanie Vaughn	USEPA	212.637.3914	vaughn.stephanie@epa.gov
Chuck Nace	USEPA	212.637.4164	nace.charles@epa.gov
Sharon Budney	CDM	732.590.4662	budneysl@cdm.com
George Molnar	CDM	908.420.8208	molnargc@cdm.com
Robert Law	de maximis, inc.	908.735.9315	rlaw@demaximis.com
Lisa Saban	Windward Environmental	206.812.5429	lisas@windwardenv.com
Mike Johns	Windward Environmental	206.812.5418	mikej@windwardenv.com
Karen Tobiason	Windward Environmental	206.812.5420	karent@windwardenv.com
Thai Do	Windward Environmental	206.812.5407	thaid@windwardenv.com

Conference call between USEPA and CPG to discuss results from the caged bivalve pilot study	
Purpose/Decisions:	A conference call between USEPA and CPG to discuss results from the caged bivalve pilot study was held on January 20, 2011. The call was held specifically to discuss the condition of the ribbed mussel shells at the end of the 45-day study because USEPA was concerned about the abrasion noted on the mussels shells and that the organisms would not survive a 90-day exposure if used for the study anticipated to begin in March 2011. During the conference call, Windward showed a Microsoft PowerPoint® presentation with photographs taken during the caged bivalve pilot study. Photos from the study showed the following: • Photos taken during the pilot study show the wear on the mussel shells observed on Day 45 was present at deployment (Day 0), and no difference in wear was detectable between deployment, Day 22, and study termination on Day 45.

QAPP Worksheet No. 9. Project Scoping Session Participants Sheet

	• Photos indicate that these abrasions are typical of mussels in general. • Photos showing dead oysters from the upper location. USEPA oversight (George Molnar) agreed that oysters were dead (approximately 39% survival) at the upper location. USEPA and CPG agreed that oysters may not be an appropriate organism to use at the more less saline locations. There was general discussion of whether ribbed mussels should be used in place of oysters (CPG preference) or whether both oysters and mussels should be used in the lower part of the river, depending on the salinity. USEPA indicated they would discuss with the Partner Agencies and get back to the CPG.

QAPP Worksheet No. 9. Project Scoping Session Participants Sheet

Project Name:	Lower Passaic River Restoration Project (LPRRP) ERA and HHRA
Site Name:	LPRSA
Projected Date(s) of Sampling:	March 2011
Site Location:	LPRSA
Project Manager:	Bill Potter/Robert Law, de maximis, inc.
Date of Session:	January 24, 2011
Purpose of Session:	Following the submittal of the caged bivalve pilot study results, USEPA and CPG held a series of discussions to finalize components of the caged bivalve study

Participants: USEPA, de maximis, inc., Windward

Name	Affiliation	Phone No.	E-mail Address
Stephanie Vaughn	USEPA	212.637.3914	vaughn.stephanie@epa.gov
Chuck Nace	USEPA	212.637.4164	nace.charles@epa.gov
Robert Law	de maximis, inc.	908.735.9315	rlaw@demaximis.com
Mike Barbara	mab Consulting	973.543.5608	mab.consulting@verizon.net

Call between USEPA and CPG to finalize components of the caged bivalve study

Decisions:

- USEPA and CPG discussed that oysters may not be an appropriate organism to use at the less saline locations in the upper portion of the estuarine zone due to the significant mortality (61%) of oysters during the caged bivalve pilot study. Based on results from the pilot study as well as historical ribbed mussel data from the study area and the low salinity conditions that are likely to exist in the river during much of the spring 2011 study, CPG strongly believes that ribbed mussels are clearly the correct species to use for the caged bivalve study. There was some discussion on whether both oysters and mussels should be used in the lower portion (higher saline portion) of the river. USEPA indicated they were leaning towards not using oysters in the LPRSA study. However, the Partner Agencies may still request Eastern oysters be deployed at locations in the lower portion of the estuarine zone.
- USEPA indicated the caged bivalve study data will not be used in the HHRA and will only require that the data be used in the uncertainty section of the BERA.
- USEPA indicated that no quantitative evaluation of the data is required.
- USEPA requested one location every 1.5 river miles, thus increasing the number of locations from 8 to 12.

QAPP Worksheet No. 10. Problem Definition

The problem to be addressed by the project:

The *in situ* caged bivalve study is being conducted at the direction of USEPA. The study is being conducted to determine the potential for caged bivalves to be used as a long-term tool for monitoring chemicals near the bottom of the water column prior to and following remediation in the LPRSA. In addition, per USEPA direction, chemical concentrations in tissue from caged bivalves will be used to assess the effects of LPRSA chemicals on bivalves and as a component in a food web model.

The environmental questions being asked:

The specific questions for the caged bivalve study addressed in this addendum is: “Are caged bivalves a viable long-term monitoring tool for monitoring chemicals in the water column prior to and following remediation in the LPRSA?,” “Are chemical of potential ecological concern (COPEC) residues in benthic invertebrate tissues from the LPRSA at levels that might cause an adverse effect on the survival, growth, and/or reproduction of mollusk populations in the LPRSA?,” and “Are COPEC residues in benthic invertebrate/caged bivalve tissues from the LPRSA at levels that might cause an adverse effect on the survival, growth, and/or reproduction of upper-level consumer populations in the LPRSA?”

The rationale for sample location:

Twelve sampling locations (Figure 1) have been selected based on the following assumptions:

- Sampling locations are representative of both estuarine and freshwater zones, as defined in the PFD (Windward and AECOM 2009). The estuarine zone includes both the brackish and transition portions of the river from River Mile (RM) 0 to RM 10, and the freshwater zone includes the freshwater portion of the river from RM 10 to RM 17.4.
- Sampling locations are spatially distributed to allow an evaluation of the LPRSA based on:
    - Locations positioned approximately every 1.5 miles.
    - No sampling locations in the vicinity of combined sewer overflows (CSOs) because the potential contributions associated with CSO discharges are not being evaluated
    - Sufficient water depth (-4ft mean lower low water [MLLW]) so that the caged bivalves will be submerged for the duration (90 days) of the exposure period
    - Low likelihood of being disturbed (e.g., not in the middle of the channel, not near docks or public access areas, near the bottom of the water column)

QAPP Worksheet No. 10. Problem Definition

The rationale for inclusion of chemical and non-chemical analyses:

The rationale for the inclusion of chemical and non-chemical analyses was presented in the Benthic QAPP (Windward 2009). However, the following modification will be implemented for this task: PAHs will be analyzed using gas chromatography/mass spectrometry – selective ion monitoring (GC/MS – SIM), rather than the high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) is described in the Benthic QAPP. When the preliminary benthic tissue data were evaluated, the majority of the detected concentrations were above the GC/MS – SIM reporting limit of 1.0 µg/kg (Windward unpublished data). The reporting limit for HRGC/HRMS for benthic tissue ranges from 0.18 to 7.7 µg/kg. Therefore, the use of GC/MS – SIM for the PAH analysis will not result in any loss of data or a reduction in overall sensitivity and will reduce the minimum mass requirements by 10 g.

Project decision conditions:

The conditions for project decisions (i.e., those decisions that may require communication between the CPG and USEPA during the field event or sampling analysis) include, but are not limited to, the need to relocate sampling locations within the LPRSA at the time of deployment, the need to delay deployment or adjust the specific days when cages are checked because of hazardous weather conditions, the prioritization of chemical analyses if insufficient tissue is available.

At locations where high mortality is observed in the test organisms (i.e., mortality exceeds 60%), observations made during the routine checks of the bivalves will be used, in consultation with USEPA, to determine if the surviving test organisms from that location will be submitted for tissue-residue analysis. A visual inspection of the specimens will be conducted and recorded.

A pre-homogenization minimum tissue mass of 105 g (post-homogenization mass 95 g), per sample, is needed for the analysis of all proposed chemical groups. The 10-g difference between pre- and post-homogenization minimum tissue mass provides an allowance for tissue loss during processing. The minimum mass requirement per chemical group is provided in the priority list below. Mass requirements have been optimized at each analytical laboratory such that these are the lowest mass requirements necessary to achieve the detection limits presented in Worksheet No. 15. The minimum mass does not include any mass required for re-extractions or matrix-specific quality control samples. Two tissue samples, one of each species, will include an additional 195 g to be used for quality assurance/quality control (QA/QC) purposes. Two samples, one of each species, will have additional mass to meet USEPA's 10% split sample objective. Per USEPA request, one sample will have an additional 320 g for sample and field duplicate analysis, and another will have an additional 480 g for sample and matrix spike/matrix spike duplicate analysis. If a post-homogenization minimum tissue mass of 95 g is not obtained, the following priority list (which is consistent with the

QAPP Worksheet No. 10. Problem Definition

USEPA-approved benthic tissue analysis plan (Windward 2010a) for the chemical analysis of tissue samples will be considered:

1. Lipids (5-g minimum mass)
2. Percent moisture (5-g minimum mass)
3. Polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (10-g minimum mass)
4. Polychlorinated biphenyl (PCB) congeners (10-g minimum mass)
5. Total and methylmercury (10-g minimum mass)
6. Organochlorine pesticides (10-g minimum mass)
7. Metals (including butyltins (15-g minimum mass)
8. PAHs (10-g minimum mass)
9. Semivolatile organic compounds (including phthalates) (10-g minimum mass)
10. PCB Aroclors(10-g minimum mass)
11. Alkylated PAHs (no additional mass; alkylated PAHs will be analyzed with the PAHs)

QAPP Worksheet No. 11. Project Quality Objectives/Systematic Planning Process Statements

What will the data be used for?

The data collected during the *in situ* caged bivalve study will be used to evaluate the potential for caged bivalves to be used as a long-term monitoring tool of chemicals in the water column of the LPRSA. In addition, per USEPA direction, chemical concentrations in tissue caged bivalves will be used to assess the effects of LPRSA chemicals on bivalves and as a component in a food web model.

What types of data are needed?

The types of data needed include:

- At deployment, physical measurements (i.e., length) of each bivalve deployed and water quality measurements (i.e., salinity/conductivity, turbidity, dissolved oxygen, pH, and temperature) from every location
- At periodic checks, physical measurements (i.e., length) for a subset of individual bivalves (approximately 10%), water quality measurements (i.e., salinity/conductivity, turbidity, dissolved oxygen, pH, and temperature) from each location and the number of dead organisms (if any) removed from each cage
- At test termination, physical measurements (i.e., length) of each bivalve from every location, survival numbers, and water quality measurements (i.e., salinity/conductivity, turbidity, dissolved oxygen, pH, and temperature) from every location
- Chemical analysis results from one tissue composite sample per location (for a total of twelve composite samples) at the end of the exposure period
- Chemical analysis results from one tissue composite sample per species (for a total of two composite samples) set aside at deployment (T_0 sample) and analyzed with tissue samples at the end of the exposure period

Note: The analytes and analytical requirements for the chemical tissue analysis are presented on Worksheet No.15.

Matrix

Chemical analysis will be conducted on whole body bivalve composite tissue samples (soft tissue, excluding the shell).

How many data are needed?

The caged bivalve study data will include one tissue composite sample from each of the twelve locations (5 locations in the freshwater zone and 7 in the estuarine zone). Two QA/QC samples, one for each species (i.e., a freshwater species and an estuarine species) will also be collected at the end of the exposure period from additional bivalves at two cage locations (one in the freshwater

QAPP Worksheet No. 11. Project Quality Objectives/Systematic Planning Process Statements

zone and one in the estuarine zone). To accommodate USEPA's request for a 10% sample split, additional bivalves will be included at two locations (one in the freshwater zone and one in the estuarine zone) for two USEPA split samples. Water quality measurements (i.e., salinity/conductivity, turbidity, dissolved oxygen, pH, and temperature) will be recorded at each location during deployment, interim monitoring events, and test termination.

Where, when, and how should the data be collected/generated?

The twelve locations where caged bivalves will be deployed are distributed at approximately 1.5 mile intervals throughout the LPRSA. The sampling locations selected for the caged bivalve study (and the rationale for each location) are presented in Worksheet No. 18 of this QAPP addendum and shown in Figure 1. The deployment of the caged bivalves must occur by early March 2011 to ensure that the study is completed prior to the summer spawning season. The next window during which the study could be conducted would be late summer 2011. Methods for the caged bivalve study will follow the SOPs detailed in Attachment U: SOP—Deployment, Monitoring and Sample Field Processing for the Caged Bivalve Study.

Who will collect and generate the data?

Windward will provide the field sampling coordination and most of the field personnel required to conduct the caged bivalve study. Windward will be supported by its contractors Aqua Survey, Inc., and the various analytical laboratories, as well as de maximis, inc., as required.

How will the data be reported?

Updates will be communicated (e.g., via telephone conversation, e-mail) to CPG project managers and project coordinators. An electronic database that includes the coordinates for the sampling locations, sampling times, sampling depths, bivalve length, survival numbers, and analytical tissue results from each location will be maintained. Preliminary data will be available upon request.

A data report that summarizes the caged bivalve study field procedures and analytical tissue results will be provided within 90 working days after the completion of the data validation of the analytical tissue results. The data report will summarize any modifications to the proposed sampling plan outlined in this QAPP addendum.

How will the data be archived?

Data records, forms, and notes will be scanned and stored electronically in a project file. Hard copies will be archived at Windward's main office in Seattle, Washington. Similarly, once the data report has been issued, it will be archived electronically.

QAPP Worksheet No. 12. Measurement Performance Criteria Table

Matrix	Tissue				
Analytical Group[a]	PAHs (and alkylated PAHs)				
Concentration Level	Low				
Sampling Procedure[b]	**Analytical Method/SOP**[b]	**Data Quality Indicators (DQIs)**	**Measurement Performance Criteria**	**QC Sample and/or Activity Used to Assess Measurement Performance**	**QC Sample Assesses Error for Sampling (S), Analytical (A) or both (S & A)**
Tissue: W	USEPA SW-846 8270C/X, Y	Accuracy/bias, contamination	No target compound > QL	Method blank/ instrument blank	A
	USEPA SW-846 8270C/X, Y	Accuracy/bias, contamination	No target compound > QL	Equipment rinsate blank[c]	S & A
	USEPA SW-846 8270C/X, Y	Accuracy/bias	Percent recovery = 50 to 130%	LCS	A
	USEPA SW-846 8270C/X, Y	Precision	RPD ≤ 30% for target compound > 5 x QL	MD	S & A
	USEPA SW-846 8270C/X, Y	Accuracy/bias, precision	Percent recovery = 50 – 130%, RPD ≤ 30%	MS/MSD	S & A
	USEPA SW-846 8270C/X, Y	Accuracy/bias	50 to 200% of the daily CCV area for the internal standards	Pre-extraction internal standards	A
	USEPA SW-846 8270C/X, Y	Completeness	≥ 90%	Data completeness check	S & A

Note: Measurement performance criteria for PAHs have been included in this addendum because of the change to a low-resolution method. Information on all other analytes is provided in the Benthic QAPP (Windward 2009).

[a] Refer to Worksheet No. 15 for a complete list of analytes for each analytical group.

[b] Reference letter is from Worksheet No. 23 for analytical SOPs and tissue sampling.

[c] Rinsate blank will be created from the homogenization equipment.

CCV – continuing calibration verification
LCS – laboratory control sample
MD – matrix duplicate
MS – matrix spike
MSD – matrix spike duplicate
PAH – polycyclic aromatic hydrocarbon
QAPP – quality assurance project plan
QC – quality control
QL – quantitation limit
RPD – relative percent difference
SOP – standard operating procedure
USEPA – US Environmental Protection Agency

QAPP Worksheet No. 13. Secondary Data Criteria and Limitations Table

Secondary Data	Data Source (originating organization, report title and date)	Data Generator(s) (originating organization, data types, data generation/collection dates)	How Data Will Be Used	Limitations on Data Use
Caged bivalve data	Caged bivalve study, Tierra Solutions (2003)	Tierra Solutions. Caged bivalve (*Geukensia demissa)* study in LPRSA, and in reference areas. Summer and fall 1999.	Locations, species and sample design will be used to support this study.	The survey focused on the lower portion of the LPRSA.
	Caged Bivalve Pilot Study Results Memorandum. January 20, 2011, Windward (2011) (Attachment Z)	Windward Environmental. Mortality and health of Eastern oysters and ribbed mussels, November 2010-January 2011	Results were used to determine if Eastern oyster could be used in this study in the upper portion (i.e., lower saline portion) of the estuarine zone. Results from the pilot study indicated that the Eastern oyster has low tolerance for low-salinity conditions, as demonstrated by their significant mortality (61%) in the upper location, as opposed to the ribbed mussel, which had a 100% survived rate at the same location. The results of the test indicated that the Eastern oyster is not a viable species for use in the upper portion of the estuarine zone.	The pilot study was designed to assess the viability of the Eastern oyster as a test species in the estuarine zone of the LPRSA.

QAPP Worksheet No. 15. Data Quality Levels and Analytical Methods Evaluation

Matrix: Tissue

Analytical Group, Method, and Laboratory: PAHs, USEPA SW-846 8270C, Alpha Analytical, Mansfield, MA

SOP from Worksheet No. 23: X, Y

Concentration Level: Low

Analyte	CAS Number	DQL (mg/kg ww)[a]	Project Quantitation Limit Goal (mg/kg ww)	Analytical Method[b]		Achievable Laboratory Limits[c]	
				MDL (mg/kg ww)	Method QL (mg/kg ww)	MDL (mg/kg ww)	QL (mg/kg ww)
2-Methylnaphthalene	91-57-6	337	0.001	NA	NA	0.000192	0.001
Acenaphthene	83-32-9	0.24[d]	0.001	NA	NA	0.000082	0.001
Acenaphthylene	208-96-8	0.24[d]	0.001	NA	NA	0.000085	0.001
Anthracene	120-12-7	0.24	0.001	NA	NA	0.000061	0.001
Fluorene	86-73-7	0.24[d]	0.001	NA	NA	0.000095	0.001
Naphthalene	91-20-3	0.24[d]	0.001	NA	NA	0.000412	0.001
Phenanthrene	85-01-8	0.24[d]	0.001	NA	NA	0.000145	0.001
Benzo[a]anthracene	56-55-3	0.24[d]	0.001	NA	NA	0.000103	0.001
Benzo[a]pyrene	50-32-8	0.24[d]	0.001	NA	NA	0.000119	0.001
Benzo[b]fluoranthene	205-99-2	0.24[d]	0.001	NA	NA	0.000146	0.001
Benzo[e]pyrene	192-97-2	NA[e]	0.001	NA	NA	0.000082	0.001
Benzo[g,h,i]perylene	191-24-2	0.24[d]	0.001	NA	NA	0.000158	0.001
Benzo[k]fluoranthene[f]	207-08-9	0.24[d]	0.001	NA	NA	0.000134	0.001
Chrysene	218-01-9	0.24[d]	0.001	NA	NA	0.000084	0.001
Dibenzo[a,h]anthracene	53-70-3	0.24[d]	0.001	NA	NA	0.000142	0.001
Fluoranthene	206-44-0	0.24[d]	0.001	NA	NA	0.000105	0.001
Indeno-[1,2,3c,d]pyrene	193-39-5	0.24[d]	0.001	NA	NA	0.000103	0.001
Perylene	198-55-0	NA[e]	0.001	NA	NA	0.000105	0.001
1-Methylnaphthalene	90-12-0	937	0.001	NA	NA	0.000167	0.001
1-Methylphenanthrene	832-69-9	NA[e]	0.001	NA	NA	0.000089	0.001
2,3,5-Trimethylnaphthalene	2245-38-7	NA[e]	0.001	NA	NA	0.000078	0.001
2,6-Dimethylnaphthalene	581-42-0	NA[e]	0.001	NA	NA	0.000129	0.001

QAPP Worksheet No. 15. Data Quality Levels and Analytical Methods Evaluation

Analyte	CAS Number	DQL (mg/kg ww)[a]	Project Quantitation Limit Goal (mg/kg ww)	Analytical Method[b]		Achievable Laboratory Limits[c]	
				MDL (mg/kg ww)	Method QL (mg/kg ww)	MDL (mg/kg ww)	QL (mg/kg ww)
Dibenzothiophene	132-65-0	293	0.001	NA	NA	0.000077	0.001
Pyrene	129-00-0	0.24[d]	0.001	NA	NA	0.000014	0.001

Notes: Project data will be reported in units appropriate to the analytical method. Data quality levels and analytical methods evaluation have been included in this addendum for PAHs because of the change to a low-resolution method. Information on all other analytes is provided in the Benthic QAPP (Windward 2009).

[a] DQLs have not been approved by USEPA. DQLs are based on the lowest available ecological tissue thresholds from tissue-residue TRVs (if available), including TRVs derived for the protection of benthos and fish as well as dietary TRVs for the protection of wildlife receptors. See Attachment K (Windward 2009) for the benthos, fish, and wildlife thresholds used to derive DQLs. DQLs (including ecological thresholds presented in Attachment K) are very conservative, generic analytical goals used solely for the purpose of evaluating laboratory analytical methods and achievable laboratory limits; these are not project-specific screening levels or PRGs. These values will be developed in subsequent phases of the project.

[b] Analytical MDLs and QLs are those documented in validated methods. When the method did not publish a value for either the MDL or the QL, the value was determined to be NA.

[c] Achievable MDLs and QLs are limits that an individual laboratory can achieve when performing a specific analytical method. Actual MDLs and QLs will vary based on sample-specific factors, including sample mass.

[d] The DQL for this analyte was based on the DQL for anthracene. DQLs have not been approved by USEPA.

[e] A DQL or project quantitation limit goal could not be established because no toxicity thresholds were available.

[f] Benzo[k]fluoranthene will be reported by the laboratory with a C-qualifier, indicating that it co-elutes with benzo[j]fluoranthene.

CAS – Chemical Abstract Service
DQL – data quality level
MDL – method detection limit
NA – not available
PAH – polycyclic aromatic hydrocarbon
PRG – preliminary remediation goal
QL – quantitation limit
TRV – toxicity reference value
ww – wet weight
USEPA – US Environmental Protection Agency

QAPP Worksheet No. 16. Project Schedule/Timeline Table

Activities	Organization	Date (MM/DD/YY)		Deliverable	Deliverable Due Date
		Anticipated Date of Initiation	Anticipated Date of Completion		
Prepare Benthic QAPP Addendum No. 4 and submit to USEPA	Windward	11/15/10	02/08/11	Benthic QAPP Addendum No. 4	02/08/11
Conduct caged bivalve study (90 days)	Windward	03/09/10	06/7/11	See below	See below
Collect T_0 bivalves, prepare and ship to Alpha Analytical	Windward	03/09/11	03/10/11	See below	See below
Retrieve cages at test termination (T_{90}), measure bivalves, prepare and ship bivalves to Alpha Analytical	Windward	06/07/11	06/09/11	See below	See below
Homogenize tissue for chemical analysis	Alpha Analytical	Upon receipt of bivalves from Windward and USEPA-approval	4 weeks after initiation of tissue homogenization	Homogenized tissue	Within 1 week of completing tissue homogenization
Analyze bivalve tissue	Alpha Analytical, Analytical Perspectives, Brooks Rand Labs, CAS, Kelso, and Maxxam Analytics	Upon receipt of homogenized tissue samples from Alpha Analytical	9 weeks after receipt of homogenized tissue samples	Final laboratory data reports and EDD	9 weeks after receipt of last bivalve tissue samples
Validate bioaccumulation tissue for chemical analysis	Laboratory Data Consultants	Upon receipt of final laboratory data reports	30 days after receipt of final laboratory data report	Final validation report	30 days after receipt of validated data
Prepare and deliver caged bivalve study data report to USEPA	Windward	Upon completion of data validation	90 days after completion of data validation	Caged bivalve study data report	90 working days after completion of data validation

CAS – Columbia Analytical Services, Inc.
EDD – electronic data deliverable
QAPP – quality assurance project plan
USEPA – US Environmental Protection Agency

QAPP Worksheet No. 18. Proposed Sampling Locations/SOP Requirements Table

Sampling Location/ID Number[a]	Easting (X)[b]	Northing (Y)[b]	River Mile	Reach[c]	Zone[d]	Bivalve Species	Rationale for Sampling Location
LPR1XX	597611	688930	1.0	1	Estuarine	Ribbed mussel (*Geukensia demissa*)	Equal spatial distribution of locations; in the vicinity of previous Tierra Solutions sampling locations in the lower 7 miles of the LPRSA (Tierra Solutions 1999); subtidal (at least -2 ft MLLW); not in the vicinity of CSOs, tributaries, docks, or other public-access areas.
LPR2AD	597839	694670	2.2	2	Estuarine		
LPR3SS	588638	692621	4.2	3	Estuarine		
LPR4ZZ	585371	701345	6.3	4	Estuarine		
LPR4AB	587961	706560	7.4	4	Estuarine		Equal spatial distribution of locations in the LPRSA; subtidal (at least -2 ft MLLW), not in the vicinity of CSOs, tributaries, docks, or other public-access areas.
LPR5XX	590692	713549	8.9	5	Estuarine		
LPR5YY	591945	718223	9.9	5	Estuarine		
LPR6UU	592107	721417	10.5	6	Freshwater	Freshwater mussel (*Elliptio complanata)*	Equal spatial distribution of locations in the LPRSA; subtidal (at least -2 ft MLLW), not in the vicinity of CSOs, tributaries, docks, or other public-access areas.
LPR7ZZ	596539	729247	12.4	7	Freshwater		
LPR7AB	597246	734894	13.6	7	Freshwater		
LPR8AC	600751	738194	15.3	8	Freshwater		
LPR8AD	599411	741553	16.1	8	Freshwater		

[a] For consistency within the database, the sampling location ID is the next sequential trap ID number available in the sequence for that reach after collection of fish tissue samples.

[b] New Jersey State Plane (US survey ft).

[c] The LPRSA is divided into eight reaches. Each reach is approximately 2 miles in length. Reaches 1 through 5 are estuarine and Reaches 6 through 8 are freshwater.

[d] Defined in the problem formulation document (Windward and AECOM 2009) as RM 0 to RM 10 and RM 10 to RM 17.4, for estuarine and freshwater zones, respectively)

CSO – combined sewer overflow
ID – identification
LPRSA – Lower Passaic River Study Area
MLLW – mean lower low water
RM – river mile

QAPP Worksheet No. 21. Project Sampling SOP References Table

SOP Reference Number	Title, Revision Date and/or Number	Originating Organization	Equipment Type	Modified for Project Work? (Y/N)	Comments
12[a]	SOP – LPRSA Deployment, Monitoring and Sample Field Processing for the Caged Bivalve Study	Windward	Bivalve cages and associated equipment for deployment, anchoring, and retrieval	N	Attachment U

[a] Next sequential number in the Benthic QAPP, taking into account any SOPs in Addenda Nos. 1 through 3.

LPRSA – Lower Passaic River Study Area

QAPP – quality assurance project plan

SOP – standard operating procedure

QAPP Worksheet No. 23. Analytical and Biological SOP References Table

Reference Number	Title, Revision Date, and/or Number	Definitive or Screening Data	Analytical Group	Instrument	Organization Performing Analysis	Modified for Project Work? (Y/N)
W	SOP No. OP-003, Tissue Preparation and Homogenization, Revision 3.3, 4/29/10	NA	NA	Glass or polyethylene cutting board; Black & Decker food processor with titanium small blade; Osterizer® blender with large stainless steel blades; ceramic, stainless steel, or titanium knives; Omni-GLH grinding unit with stainless steel or titanium saw tooth probes; Janke & Kunkel IKA tissuemizer	Alpha Analytical	N
X	SOP No O-008. Analysis of Parent and Alkylated Polynuclear Aromatic Hydrocarbons, Selected Heterocyclic Compounds, Steranes, Triterpanes, and Triaromatic Steroids by GC/MS – SIM, Revision 6.1, 9/23/10	Definitive	PAHs and Alkylated PAHs	GC Model Agilent/HP6890 or equivalent, Mass spectrometer Agilent/HP5973 or equivalent	Alpha Analytical	N
Y	SOP No. OP-009. Alumina Column Cleanup of Organic Extracts, Revision 1.0 4/17/08	Definitive	PAHs and Alkylated PAHs	Glass preparation column, muffle furnace, and a top-loading balance capable of weighing to the nearest 0.01 g	Alpha Analytical	N

Note: The SOPs attached to this addendum relate to the analysis of PAHs. Information on all other analytes is provided in the Benthic QAPP (Windward 2009).

GC/MS gas chromatography/mass spectrometry
NA – not applicable
PAH – polycyclic aromatic hydrocarbon
QAPP – quality assurance project plan
SIM – selective ion monitoring
SOP – standard operating procedure

QAPP Worksheet No. 24. Analytical Instrument Calibration Table

Instrument – Chemical	Calibration Procedure	Frequency of Calibration	Acceptance Criteria	Corrective Action (CA)	Person Responsible for CA	SOP Reference[a]
GC/MS-SIM – PAHs and alkylated PAHs	Alpha Analytical SOP No. O-008	Initial calibration before analysis of sample extracts, initial calibration check standard (CCC) following calibration curve; CCV at the beginning and end of every analytical sequence and every 24 hours within the sequence	ICAL: All recoveries must be ±25% of true values. CCV: Compare the CCV resulting response against the average response for the initial calibration for each calibrated PAH; the percent difference for each calibrated PAH must be < 25%, with no more than 10% of all compounds > 25% but < 35%	Inspect system, correct problem, rerun calibration and affected samples.	Analyst or Susan O'Neil or Andrew Cram, Alpha Analytical	X

Note: The analytical instrument calibration information included in this addendum relate to the analysis of PAHs. Information on all other analytes is provided in the Benthic QAPP (Windward 2009).

[a] From Worksheet No. 23.

CCV – continuing calibration verification
GC/MS – gas chromatography/mass spectrometry
ICAL – initial calibration
PAH – polycyclic aromatic hydrocarbon
RSD – relative standard deviation
SIM – selective ion monitoring

QAPP Worksheet No. 27. Sample Custody Requirements Table

Sample Identification Procedures:

The caged bivalve tissue composite samples will be documented with the site name, time, date, sampling location, and field crew initials. Unique alphanumeric identification (ID) numbers will be assigned to each individual bivalve as well as each bivalve tissue composite sample. The sample identification scheme is as follows:

The individual specimens will be identified as follows:

- The first seven characters, the location ID, will be "LPR" to identify the project area (Lower Passaic River) followed by the numbers 1 to 8 to identify the 2-mile reach, and then followed by two letters identifying the next Trap ID in the sequence for that reach, as shown in Worksheet No 18.
- The next set of alphanumeric characters will be the specimen ID to identify the bivalve species by its scientific name: GD for *Geukensia demissa,* or EC for *Elliptio complanata* and a three-digit sequential number of the specimen captured within the sampling area.
- For example, the first *Geukensia demissa* from the first trap in Reach 1 (RM 0 to RM 2) would be identified as "LPR1XX-GD001

The individual specimens will be composited from each location before chemical analysis and the sample identification scheme is as follows:

- The first five characters will be "LPR" to identify the project area (Lower Passaic River) and compositing area (i.e., the 2-mile reach [1 to 8]
- The next set of alphanumeric characters will identify the each bivalve species by its scientific name: GD for *Geukensia demissa,* or EC for *Elliptio complanata*
- The next set of characters will be "ST" to identify the sample as soft tissue.
- The next set of alphanumeric characters will be "Comp" to identify the composite sample, followed by a two-digit sequential number within the sampling area.
- For example, *a Geukensia demissa* composite tissue sample from the first trap (LPR1XX) in the first 2-mile reach of the LPR would be identified as LPR1-GDST-Comp01 and the second trap (LPR1YY) would be identified as LPR1-GDST-Comp02.

QAPP Worksheet No. 28. QC Samples Table

Matrix	Tissue
Analytical Group	PAHs (and alkylated PAHs)
Concentration Level	Low
Sampling SOP[a]	Attachment U
Analytical Method/SOP Reference[a]	USEPA SW-846 8270C/X,Y
Sampler's Name	Windward Field Staff
Field Sampling Organization	Windward Environmental LLC
Analytical Organization	Alpha Analytical
Number of Sampling Locations	Tissue: 12

QC Sample	Frequency/ Number	Method/SOP QC Acceptance Limits	Corrective Action	Person(s) Responsible for Corrective Action	Data Quality Indicator (DQI)	Measurement Performance Criteria
Method blank/instrument blank	1 per batch of 20 samples	No target compounds > QL	Flag associated results if detected and/or greater than 1/10 of the amount found in samples.	Susan O'Neil (or alternate analyst), Alpha Analytical	Contamination	No target compounds > QL
MS/MSD	1 per batch of 20 samples per matrix type (mass permitting)	Percent recovery = 50 – 150%, RPD ≤ 30%	Flag associated results.	Susan O'Neil (or alternate analyst), Alpha Analytical	Precision	Laboratory recovery and RPD control limit
MD	1 per batch of 20 samples per matrix type (mass permitting)	RPD ≤ 30% if target compounds are > 5 x QL	Flag associated results.	Susan O'Neil (or alternate analyst), Alpha Analytical	Precision	Laboratory recovery and RPD control limit
Pre-extraction internal standard	Added to every sample and QC sample	50 – 200% of the daily CCV area for the internal standards	Refer to SOP for corrective action.	Susan O'Neil (or alternate analyst), Alpha Analytical	Accuracy	Laboratory recovery limits

QAPP Worksheet No. 28. QC Samples Table

QC Sample	Frequency/ Number	Method/SOP QC Acceptance Limits	Corrective Action	Person(s) Responsible for Corrective Action	Data Quality Indicator (DQI)	Measurement Performance Criteria
LCS	At the beginning and end of the 12-hour analytical period	Percent recovery = 50 – 130%	Re-analyze affected samples.	Susan O'Neil (or alternate analyst), Alpha Analytical	Precision/ accuracy	Laboratory RPD control limit and percent drift

Note: The QC sample information included in this addendum relate to the analysis of PAHs. Information on all other analytes is provided in the Benthic QAPP (Windward 2009).

[a] From Worksheet No. 23.

CCV –continuing calibration verification
LCS – laboratory control sample
MD – matrix duplicate
MS – matrix spike
MSD – matrix spike duplicate
PAH – polycyclic aromatic hydrocarbon
QAPP – quality assurance project plan
QC – quality control
QL – quantitation limit
RPD – relative percent difference
SOP – standard operating procedure
USEPA – US Environmental Protection Agency

QAPP Worksheet No. 29. Project Documents and Records Table

Survey Documents and Records
Onsite Analysis Documents and Records
Caged Bivalve Study Data Form (Attachment V)
Deliverables
Caged bivalve study data report

References

Tierra Solutions. 1999. Passaic River Study Area ecological sampling plan. Work plan/field sampling plan. Volume 1 of 6. Tierra Solutions, Inc., Newark, NJ.

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Windward. 2010a. Memorandum dated June 14, 2010, to Robert Law, de maximis, inc.: Benthic tissue analysis plan. Lower Passaic River Restoration Project. Lower Passaic River Study Area RI/FS. Windward Environmental LLC, Seattle, WA.

Windward. 2010b. Memorandum dated November 16, 2010, to de maximis, inc.: Caged bivalve study. Lower Passaic River Restoration Project. Lower Passaic River Study Area RI/FS. Windward Environmental LLC, Seattle, WA.

Windward. 2011. Memorandum dated January 20, 2011, to Cooperating Parties Group: Caged bivalve pilot study results. Lower Passaic River Restoration Project. Lower Passaic River Study Area RI/FS. Windward Environmental LLC, Seattle, WA.

Windward, AECOM. 2009. LPRSA human health and ecological risk assessment streamlined 2009 problem formulation. Final. Prepared for Cooperating Parties Group, Newark, New Jersey. Windward Environmental LLC, Seattle, WA; AECOM, Inc., Westford, MA.

Attachment U: SOP—LPRSA Deployment, Monitoring, and Sample Field Processing for the Caged Bivalve Study

I. Purpose

The purpose of this standard operating procedure (SOP) is to define the procedures to be followed for conducting the Lower Passaic River Study Area (LPRSA) caged bivalve study, specifically the equipment required and the field methods necessary for the deployment, monitoring, and sample field processing of caged bivalves.

II. Preparation for Sampling

This SOP identifies the bivalve species; the handling procedures, exposure duration, monitoring procedures, and equipment for the caged bivalve study. The field team is responsible for reviewing the QAPP prior to conducting field activities and ensuring that all field equipment is available and in acceptable condition.

III. Equipment and Supplies

- Bivalve holding cages
- Mesh netting
- Buoys and rope
- Holding cage anchoring:
    - Cinder blocks
    - Zip ties
    - Rope
- Vessel for deployment, monitoring, and retrieval
- Bivalves from Aquatic Research Organisms, Inc. (ARO)
    - Freshwater mussel (*Elliptio complanata)*
    - Ribbed mussel (*Geukensia demissa*)
- Digital camera
- Global positioning system (GPS) unit (specifications and procedures are in Attachment B: SOP—Locating Sample Points Using a Hand-Held Global Positioning System (GPS) and Attachment C: SOP—Locating Sample Points Using a Boat-Mounted Global Positioning System (GPS) of the Benthic QAPP (Windward 2009)
- Site maps
- Tide tables
- Field forms, log book, waterproof pens, pencils, and grease pencils
- Batteries for cameras (size will depend on the device)
- Stainless steel spoon

- Electronic scale
- Aluminum foil
- Labels
- Chain of custody forms
- Ziplock bags
- Bubble wrap
- Coolers
- Ice

IV. Bivalve selection and preparation

Two types of bivalves will be used based on whether the deployment location is in the freshwater or estuarine zone. A freshwater mussel (*Elliptio complanata)* will be used in the freshwater zone; the ribbed mussel (*Geukensia demissa*) will be used in the estuarine zone. A 45-day caged bivalve pilot study was conducted in the winter of 2010 to evaluate the viability of the Eastern oyster (*Crassostrea virginica*) for use as the estuarine species for this study. In the pilot study, the Eastern oyster had 61% mortality at the upper location, where salinity was less than 6 parts per thousand, whereas all of the ribbed mussels survived at the same location (Windward 2011). Because of a New Jersey Department of Environmental Protection ban on growing commercial shellfish in contaminated waters for research and restoration purposes, Eastern oysters will not be deployed in the lower portion of the estuarine zone. Thus, the ribbed mussel was selected as the most appropriate bivalve species for use in the estuarine portion of the LPRSA for this caged bivalve study. The bivalves will be adults of similar size and sourced from ARO, which is located in Hampton, New Hampshire. The bivalves will be acclimated to site temperature and salinity by ARO for a minimum of 3 days prior to deployment. Upon receipt of the bivalves from ARO, two of each bivalve species will be randomly selected and sacrificed for an initial health exam to verify that the organisms are healthy and suitable for use in the caged bivalve study. On Day 0 (day of deployment), sufficient numbers of organisms to create a time 0 sample (T_0) for each species will be set aside for the analysis of baseline conditions. T_0 samples will be measured (for length) prior to being submitted for chemical analysis. These bivalves will be transported to Alpha Analytical, where they will be frozen and held until the caged bivalve study is completed and tissue from the full study is ready to be processed for analysis.

Sufficient numbers of bivalves (52 mussels) will be added to each cage in order to achieve a minimum pre-homogenization mass of 105 g wet weight, and to account for potential bivalve mortality and periodic sacrifice for health inspections. At two sampling locations (one per zone), additional bivalves will be added to meet the requirements for USEPA split samples. Based on USEPA's request for 320 g from one location for sample and field duplicate analysis and 480 g from a second location for sample and matrix spike/matrix spike duplicate analysis, an additional 131 mussels will be added at one location (e.g., LPR4ZZ) and 196 mussels will be added at another location (e.g., LPR7AB). Additionally, at two sampling locations (one per species/zone), additional bivalves (81 mussels) for a total of 133 mussels will be added to meet the requirements

for quality assurance/quality control (QA/QC). Table 1 presents the number of bivalves required for this study. The bivalves will be measured (for length) before deployment. The bivalves will be placed in appropriate holding containers, such as coolers, for transport to the field for deployment in order to keep the bivalves cool, wet, and away from direct sunlight.

Table 1. Number of Bivalves Required per Location for the Caged Bivalve Study

Purpose	Number of Bivalves per Sampling Location[a]
Analytical requirement (105g ww tissue)	39
Day 14 sacrifice	2
Day 35 sacrifice	2
Day 56 sacrifice	2
Day 75 sacrifice	2
10% contingency for mortality or if mean mass is less than expected	5
Total per cage at deployment (total number of each species at each location to meet minimum mass requirements)	**52**
QA/QC analytical requirement (195 g ww tissue)	73
10% contingency for mortality or if mean mass is less than expected	8
Additional bivalves for QA/QC (at one location per species [one in the freshwater zone and one in the estuarine zone])	**81[b]**
USEPA split analytical requirement (320 g ww tissue) at one location	119[c]
10% contingency for mortality or if mean mass is less than expected	12
USEPA split analytical requirement (480 g ww tissue) at one location	178[d]
10% contingency for mortality or if mean mass is less than expected	18
Additional bivalves for USEPA split samples (at one location per species [one in the freshwater zone and one in the estuarine zone])	**131 and 196[e]**

[a] Number of bivalves necessary was based on an estimated mass per bivalve of 2.7g ww. Number is rounded up to the nearest whole number.

[b] A total of 133 bivalves (52 plus 81) will be deployed at QA/QC sample locations.

[c] 320 g ww tissue are required by USEPA for split and field duplicate samples.

[d] 480 g ww tissue are required by USEPA for split and matrix spike/matrix spike duplicate samples.

[e] A total of 183 bivalves (52 plus 131) will be deployed at one USEPA split location, and 248 (52 plus 196) will be deployed at the other USEPA location.

QA/QC – quality assurance/quality control

USEPA – US Environmental Protection Agency

ww – wet weight

V. Holding Cage Design

Holding cages will be constructed using a double-cage design – an inner plastic mesh cage to contain the bivalves and a rigid outer cage made of lightweight plastic material to serve as a barrier to predators. The cages will be deployed at least 1 ft off the bottom substrate by securing the test cage to four cinderblocks that will sit on the river bottom and provide additional stability. Based on observations during the pilot study,

the cages may sink into the sediment, but not by more than 6 inches. A buoy will be attached to each cage to facilitate retrieval. Figure 1 provides a schematic for the test cage deployment configuration.

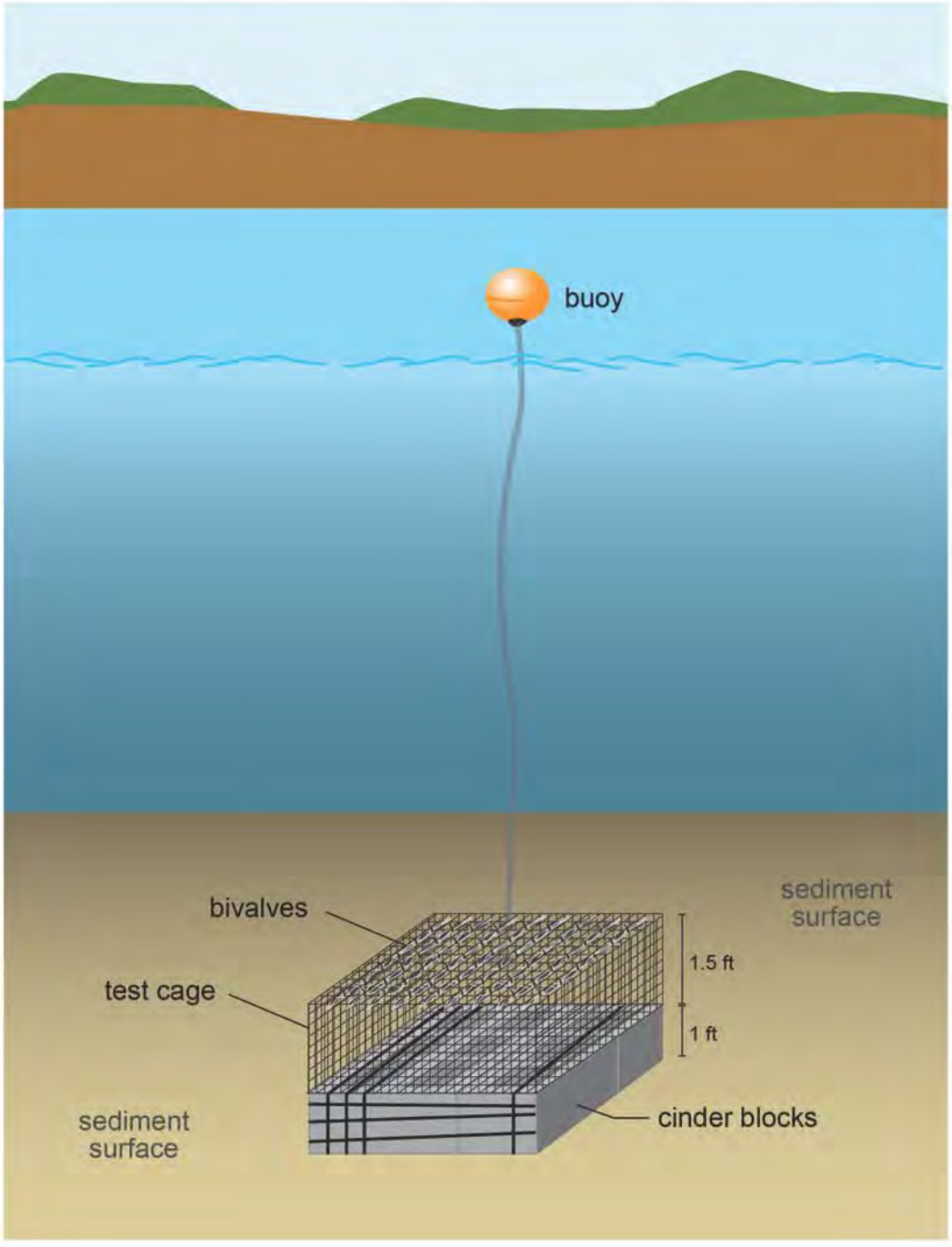

Figure 1. Schematic of bivalve cage deployment configuration

VI. Field Procedures

The caged bivalve study will be conducted by deploying, monitoring, and retrieving bivalves submerged in duplicate protective cages at twelve locations (seven in the estuarine zone and five in the freshwater zone) in the LPRSA as presented in Worksheet No. 18 of the Benthic QAPP Addendum No. 4. The caged bivalve study will be performed for 90 days. The bivalves will be monitored two weeks after deployment (Day 14), and approximately every three weeks thereafter (e.g., Day 35, Day 56, Day 75), and at test termination (Day 90) unless the amount of fouling noted on the cages during the periodic checks indicates that defouling should occur more frequently.

The bivalves will be placed into the holding cages in a single, even layer (to eliminate clumping) suspended from top of the cage. Prior to cage deployment, care will be taken to minimize the handling of organisms and to keep them moist and out of direct sunlight in order to avoid desiccation. The cages (strapped to cinderblocks) will be deployed on the bottom substrate. As the cage is lowered, any floating bivalves (indicating the presence of air within the mantle cavity) will be removed and replaced.

The cages will be deployed in water with a minimum depth of -4 ft MLLW to ensure that the bivalve cages will remain submerged throughout the duration of this study. The actual water depth at the time of deployment and cage location coordinates will be recorded. Salinity/conductivity, turbidity, dissolved oxygen, pH, and temperature measurements will be also recorded at the time of deployment.

During each monitoring event (e.g., Day 14, Day 35, Day 56, and Day 75):

- All bivalves will be visually examined. During the visual inspections, bivalves that are determined to be dead will be removed from the cages. In addition two bivalves from each cage will be removed, opened, and inspected for general tissue health. The results of the inspection will be recorded in the field log book.
- Cages will be defouled before being returned to the water.
- A subset of individuals (approximately 10%) will be measured for length.
- Water salinity/conductivity, turbidity, dissolved oxygen, pH, and temperature measurements will also be checked at each location during the monitoring and will be recorded at 1-ft intervals in the water column.

At test termination (i.e., Day 90):

- All bivalves will be visually examined and measured (for length). All results of the examination (e.g., gaping shells) will be recorded on the caged bivalve data form (Attachment V of the Benthic QAPP Addendum No. 4). Bivalves with open or severely damaged shells or a siphon tube that does not respond to touch will be considered dead. Bivalves that are determined to be dead during the visual inspection will be discarded and not submitted for tissue chemical analysis.

The bivalves from each location will be placed in labeled ziplock bags, carefully packed in coolers and sent to the laboratory, where they will be frozen as whole individuals. Alpha Analytical will be responsible for shucking and preparing the individual bivalves for homogenization as described in the in Attachment W of the Benthic QAPP Addendum No. 4, SOP—Tissue Preparation and Homogenization. Alpha Analytical will homogenize the samples as described in Attachment W and distribute the tissue

samples to the other laboratories that are responsible for conducting analyses, consistent with the Benthic QAPP (Windward 2009).

VI. References

Windward. 2009. Lower Passaic River Restoration Project. Lower Passaic River Study Area RI/FS. Quality Assurance Project Plan: Surface sediment chemical analyses and benthic invertebrate toxicity and bioaccumulation testing. Final. Prepared for Cooperating Parties Group, Newark, New Jersey. Windward Environmental LLC, Seattle, WA.

Windward. 2010. Lower Passaic River Restoration Project. Caged Bivalve Pilot Study Memorandum. Prepared for Cooperating Parties Group, Newark, New Jersey. Windward Environmental LLC, Seattle, WA.

Windward. 2011. Memorandum dated January 20, 2011, to Cooperating Parties Group: Caged bivalve pilot study results. Lower Passaic River Restoration Project. Lower Passaic River Study Area RI/FS. Windward Environmental LLC, Seattle, WA.

Attachment V: LPRSA Caged Bivalve Study Data Form

Project Name:	**Project No.:**
Date/Time:	**Field Crew Initials:**
Cage ID:	**Species:**
Pre-deployment or Post deployment (circle one)	
Comments:	

Specimen ID	Shell Length (cm)	Comments

Attachment W: SOP—Tissue Preparation and Homogenization

Tissue Preparation and Homogenization

References: This standard operating procedure (SOP) is a performance-based method. This SOP describes the procedure as developed by Alpha Analytical.

Uncontrolled Document

Copy No.: _____

Prepared By:

Name: Normand Laurianno Position: Extractions/ Forensics Analytical Team Leader

Signature: _____ Date: 04/29/2010

Authorized By:

Name: Leonard Pitts Position: Laboratory/Technical Director

Signature: _____ Date: 4/29/10

ISSUE AMENDMENTS

Changes since last issue:

Section 2: Add of carcass as type of fish tissue.
Section 7: Addition of sections for pliers, electric grinder, glass weighing dish, camera, ruler, latex gloves – powder free, paper towels.
Section 7.3: Modification to include all dissection tools and utensils which may be utilized in processing.
Section 9.1.1: Addition of procedure for generation of Rinseate Blanks.
Section 10.1.1: Addition of generator probes.
Section 10.1.2: Removal of 'organic analysis only' statement.
Section 10.1.3: Changed to Section 10.1.2.1.
Section 10.3.4: Addition option for project-specific sample weight determinations; removal of use of aluminum foil/plastic wrap to cover balance; addition of photo of fish with ruler; clarification of tools used to skin fish.
Section 10.3.4.5: Clarification of homogenization for whole body, fillets and carcass.
Sections 10.3.5.6; 10.3.6.1.3; & 10.3.7.2: Remove "Teflon Covered Spatula".
Section 10.3.6.2.1: Add "Remove and discard eggs from female crabs".
Section 10.3.9.3.1: Addition of procedure for removal of skin for eels.
Sections 13.1 and 13.2: Modified to not applicable.

Tissue Preparation and Homogenization

References: This standard operating procedure (SOP) is a performance-based method. This SOP describes the procedure as developed by Alpha Analytical.

1. Scope and Application

Matrices: This method is applicable to the preparation and homogenization of plant and animal tissue including: mammals (mice or shrew etc.), fish (whole body and fillets), mollusks (mussels or clams, etc.), crustaceans (lobster or shrimp, etc.), reptiles and amphibians (frogs or turtles, etc.) macro invertebrates (benthic worms, eels, insects and other biota), and vegetation (coastal and wetland grasses)

Definitions: Refer to Alpha Analytical Quality Manual.

This preparation and homogenization procedure may be used prior to the extraction or digestion of the matrices listed above, for the ultimate detection of organic and inorganic analytes. Because this procedure is performance based, it should only be used for compounds where studies have assessed the precision, accuracy, and sensitivity of the technique relative to the project specific goals.

This method is intended to describe the preparation and homogenization procedures to be followed prior to the extraction, digestion and/or clean up of sample extracts or digestates. This procedure uses a variety of cutting and grinding equipment for size reduction, compositing and homogenization. See Section 7 for Equipment and Materials. This method is applicable to the matrices described above. The final determinative analytical methods and lists of potential target compounds are noted in the SOPs referenced below. Applicable extraction, digestion and cleanup methods include:

- *Microwave Assisted Acid Digestion of Sediments, Soils, Tissues and Waters* (MP-003),
- *Gel Permeation Chromatography* (OP-006),
- *Sulfuric Acid Cleanup - Method 3665A* (OP-010),
- *Microscale Solvent Extraction (OP-016*
- *Alumina Column Cleanup (OP-009)*.

Other applicable methods, however not included by ALPHA ANALYTICAL SOP reference, are Method 3546 - Microwave Extraction of Organic Compounds and Automated Silica Gel Cleanup for Organic Compounds. <u>Note:</u> *Sample cleanup does not apply to digestates of inorganic samples for metals analysis.*

Data derived from the analysis of tissue samples is generally used to determine if human health, and/or ecological risk criteria have been exceeded.

The data report packages present the documentation of any method modification related to the samples tested. Depending upon the nature of the modification and the extent of intended use, the laboratory may be required to demonstrate that the modifications will produce equivalent results for the matrix. Approval of all method modifications is by one or more of the following laboratory personnel before performing the modification: Area Supervisor, Department Supervisor, Laboratory Director, or Quality Assurance Officer.

This method is restricted to use by or under the supervision of experienced analysts.

2. Summary of Method

This method describes the tissue processing and homogenization procedures to be used prior to the extraction/digestion and analysis of the sample. Samples are best processed when partially frozen. Samples may be re-frozen after processing pending extraction or digestion.

Fish tissue samples (whole bodies, carcass or fillets) are weighed and the weights are recorded following project specifications. Measurements may be taken as needed depending upon the project specifications. The fish may be processed with the skin on or off, depending upon the project specifications. If fillets are to be removed and processed separately, this is generally done after the removal of the skin, however fillets can be processed with the skin on if requested. If compositing is required, the identified samples for composite are filleted or skinned prior to compositing homogenization. The carcass of the fish (after removal of the fillet) may be maintained for separate homogenization and analysis if requested.

Mammals such as mice, shrew or other rodents, must be prepared in a glove box due to the potential health hazards associated with mammal tissue. All project specific sample preparation (weighing, skinning, compositing and homogenization) is performed in the glove box. Waste from the processing must be containerized and treated with bleach before disposal. Waste from the processing must be containerized before disposal. The outside surfaces of the sample containers must be disinfected before removal from the glove box.

Mollusks, crustaceans and other like invertebrates are measured and weighed prior to processing. Mollusks must be removed from their shells before processing. Due to the low weight of a single mollusk, crustacean or invertebrate, these sample types are generally composited with others of the same species and/or sampling area prior to homogenization. Gender determination may need to be performed with larger crustaceans such as lobsters. This is done prior to any processing and recorded. Additionally, lobsters are usually dissected, and the edible meat (tail and claw) is removed for homogenization. Certain internal organs such as the hepatopancreas may need to be processed separately. If crabs are being processed, the legs, claws and body cavity are generally homogenized together.

Reptiles and amphibians are generally processed as whole body samples. Depending upon the size, the specimen may need to be cut into small pieces and processed in part, then re-combined as a single sample. Due to the thickness of the skin of most reptiles, such as frogs, it is recommended that these be processed without the skin. If the skin must be processed, ensure that the grinder or processor blades are sharpened before use. The blades may need to be re-sharpened between every few samples as needed. Turtles must be removed from the shell prior to processing by digging out the head and legs, and as much of the body as feasible.

Macro invertebrates such as worms, eels, insects or benthic biota are generally processed as whole body samples. Depending upon the size, the specimen may need to be cut into small pieces and processed in part, then re-combined as a single sample. Due to the low weight of a single invertebrate, these sample types are generally composited with others of the same species and/or sampling area prior to homogenization.

Plants are rinsed prior to processing to remove soil, silt, small insects or other debris. Depending upon the size of the plant and the leaves, the sample may be processed mechanically, or may have to be cut into small pieces by hand. Plants can be processed either wet or dry, depending upon project specifications.

After tissue processing, organic samples will be extracted and the extracts cleaned if needed, then analyzed by the determinative analytical procedure. Inorganic digestates do not require further clean up and will only undergo analysis by the determinative analytical procedure.

2.1 Method Modifications from Reference

None.

3. Reporting Limits

Not applicable to this method. Refer to the analytical method SOPs.

4. Interferences

Solvents, reagents, processing equipment and glassware may introduce interferences. These must be demonstrated to be free of interferences by the analysis of a method blank. See the ALPHA ANALYTICAL SOP *Reagent, Solvent and Standard Control* (G-008) and *Laboratory Glassware Cleaning* (G-002), for additional details.

Field Blanks are recommended to ensure that the field sample packing materials are not a potential source of contamination. This can be done by pouring contaminate free water over the sample collection material and collecting the water in an appropriate container with preservative as needed (*i.e.,* 1L glass amber bottle for organic and a 500mL polyethylene bottle with 1:1 HNO_3 preservative for metals).

Equipment used to process samples for *organic* analyses should be made of stainless steel, Teflon, ceramic, or PTFE. Tissue should be removed with clean, high-quality, corrosion-resistant stainless steel, ceramic or titanium instruments, knives and blades. Homogenates must be stored in borosilicate glass, quartz, or PTFE containers with PTFE-lined lids.

Many interferences can be removed by sample cleanup. The organic cleanup methods performed by ALPHA ANALYTICAL include those listed in Section 1. Only appropriate cleanup techniques must be performed based on the suspected interference and the compounds of interest. For example, sulfuric acid cleanup is not applicable to samples requiring pesticide analysis because this rigorous cleanup will destroy the majority of pesticides.

Soapy residue may result in basic conditions on glassware and may cause degradation of the pesticides Aldrin and Heptachlor, some organophosphorous pesticides, and can cause metals instrument interferences. All glassware must be rinsed thoroughly with deionized water and solvents/nitric acid to remove soapy residue. See the ALPHA ANALYTICAL SOP (G-002) *Laboratory Glassware Cleaning*, for additional details.

5. Health and Safety

The toxicity or carcinogenicity of each reagent and standard used in this method is not fully established; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. A reference file of material safety data sheets is available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available in the Chemical Hygiene Plan.

All personnel handling environmental samples known to contain or to have been in contact with municipal waste must follow safety practices for handling known disease causative agents.

6. Sample Collection, Preservation, Shipping and Handling

6.1 Sample Collection

As guidance, a minimum of 50 grams of sample must be collected for organic analyses, and 5 grams for metals analyses, in a glass jar with a Teflon or PTFE-lined screw cap. The amount of sample needed, will depend upon the project DQOs, such as reporting limits and the need for MS/MSD and/or duplicate analyses. Extra sample must be collected, if possible, to allow the laboratory adequate sample volume in case re-preparation and re-analysis is needed. Large

whole individual fish, fillets, or vegetation may be wrapped in plastic or aluminum foil depending upon the requested analyses. (See Section 4 or additional details about allowable materials). Large crustaceans, reptiles or amphibians may be individually packed in well-labeled Styrofoam coolers.

6.2 Sample Preservation

It is recommended that samples are preserved by freezing them with dry ice at ≤ -20°C. If samples are not shipped frozen, they will be stored in freezers at Alpha Analytical upon arrival, and until processing. The samples must remain frozen and maintained at ≤ -20°C ± 10°C until processing. After processing, individual sample homogenates must also be stored at ≤ -20°C ±10°C until extraction/digestion and analysis.

6.3 Sample Shipping

Refer to Section 6.2.

6.4 Sample Handling

Sample processing and extraction/digestion hold times are suspended by freezing the sample. Hold time monitoring is resumed when samples are removed from freezers for processing and then returned to freezers pending extraction/digestion. Movement of samples into and out of freezers is tracked through LIMS. The organic hold time is 14 days from sample collection to extraction, and 40 days from extraction to analysis. The metals hold time is six months from sample collection to digestion and analysis. If mercury is to be determined, the hold time is 28 days from sample collection to digestion and analysis.

7. Equipment and Supplies

7.1 Cutting board: Made of either glass or Teflon.

7.2 Food processor: Black & Decker with titanium cutting blade (small).

7.3 Dissection Tools: Tools may include the following utensils: knives/blades (ceramic, stainless steel, or titanium), stainless steel picks, spatulas (stainless steel or Teflon-coated stainless steel), stainless steel scissors/snips, stainless steel tweezers. (Refer to Section 4.0 for interferences and/or contamination associated with different materials.)

7.4 Pliers: Stainless Steel

7.5 Balances: Analytical Balance with precision to 0.0001g; Top loading balance with precision to 0.01g; Top loading balance with precision to 0.2g.

7.6 Grinding unit: Omni-GLH, electric, custom fitted with stainless steel or titanium interior saw tooth probes (10mm, 20mm, 45mm), or equivalent.

7.7 Tissuemizer: *Janke & Kunkel* IKA - Labortechnik Ultra Turrax T25, stainless steel

7.8 Grinder: LEM electric meat grinder, stainless steel (or equivalent)

7.9 Multi-hazard glove box: Labconco

7.10 Bench liner material

7.11 Latex Gloves – Powder Free

7.12 Glass weighing dish/jar, wax paper, aluminum foil, plastic wrap.

7.13 Camera

7.14 Ruler

7.15 Paper towels: Kim Wipes

8. Reagents and Standards

Use reagent grade or trace metals grade chemicals for all reagents. Deionized (DI) water or reagent water is ASTM Type II laboratory reagent grade water. Other grades may be used.

All reagents are stored at room temperature in flammable cabinets, unless otherwise noted. All reagents expire upon manufacturer's expiration date or one year from date of opening, whichever is sooner.

8.1 Methylene Chloride: ACS approved, Pesticide grade, see ALPHA ANALYTICAL SOP *Reagent, Solvent and Standard Control* (G-008) for additional details regarding solvent purity.

8.2 Methanol: ACS approved, Purge & Trap grade, see ALPHA ANALYTICAL SOP *Reagent, Solvent and Standard Control* (G-008) for additional details regarding solvent purity.

8.3 Hexane: ACS approved, Pesticide grade, see ALPHA ANALYTICAL SOP Reagent. *Solvent and Standard Control* (G-008) for additional details regarding solvent purity

8.4 Acetone: ACS approved, HPLC grade, see ALPHA ANALYTICAL SOP *Reagent, Solvent and Standard Control* (G-008) for additional details regarding solvent purity.

8.5 Nitric acid 50% (1:1): Add 500 mL concentrated HNO_3 to 400 mL of reagent water and dilute to 1 liter in an appropriate beaker or flask. For 25% HNO_3 solution: add 250 mL of concentrated HNO_3 to 400 mL of reagent water and dilute to 1 liter in an appropriate beaker or flask. Store in a corrosion-resistant cabinet.

8.6 10% Bleach solution: Add 100 mL of commercial bleach to 500 mL of reagent water and dilute to 1 liter in an appropriate beaker or flask. Prepare fresh each day of use.

8.7 Alconox cleaning solution. No special storage requirements. No expiration requirements.

9. Quality Control

The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

The following quality control samples mayor may not be included with each processing batch. If not included in the tissue processing steps, they must be included in the extraction/digestion batches that follow processing, or as needed, depending upon project specifications.

9.1 Blank(s)

9.1.1 Rinseate Blank/Equipment Blank or Process Blank

Rinseate/Equipment or Process blanks are generated using contaminate-free reagent (DI) water to rinse all processing equipment after completion of the cleaning procedure (see Section 10.1). The volume of water used will be based on project-specific volume requirements for requested analyses.

All processing equipment is rinsed with pre-determined volume of reagent water (DI) into a collection vessel. All rinse water is transferred from collection vessels to larger glass carboy.

Homogenizer/Generator probes are immersed in a pre-determined volume of DI water. The Homogenizing Unit will be turned on and the probe will process the DI water for a pre-determined time, based on project specifications. The DI water will then be transferred into a larger glass carboy and combined with DI from other processing equipment.

After all rinseates are collected into the glass carboy (or appropriate container), mix the DI water using a large glass stirring rod or by swirling the DI water. Transfer the water into the appropriate pre-prepared sample containers.

9.1.2 Method Blank

Not applicable to this method. Refer to analytical SOPs.

9.2 Laboratory Control Sample (LCS)

Not applicable to this method. Refer to analytical SOPs

9.3 Initial Calibration Verification (ICV)

Not applicable to this method. Refer to analytical SOPs.

9.4 Continuing Calibration Verification (CCV)

Not applicable to this method. Refer to analytical SOPs.

9.5 Matrix Spike

Not applicable to this method. Refer to analytical SOPs

9.6 Laboratory Duplicate

Not applicable to this method. Refer to analytical SOPs

9.7 Method-specific Quality Control Samples

Not applicable to this method. Refer to analytical SOPs

9.8 Method Sequence

Not applicable.

10. Procedure

The procedures described below are general cleaning and pre-processing procedures that are to be followed regardless of the type of tissue being processed. Samples are prioritized by the Department Manager or Team Leader based on hold time and client due date. All weights, measurement and other project required observations are recorded on the Tissue Prep Log sheets.

10.1 Equipment Set-up

10.1.1 Wash all utensils, generator probes, sample processor (blades, blade post, cup and lid) and the cutting board(s) with an alconox solution and a sponge. Rinse thoroughly with tap water, then with DI water and allow to dry. Equipment may be dried with a paper towel, if needed.

10.1.2 After drying the equipment, rinse all utensils, processor parts and surfaces with Acetone followed by a rinse with methylene chloride.

10.1.2.1 For *metal analyses only*, rinse all plastic and ceramic utensils with 25% HNO3 followed by another rinse with DI water. Rinse processor parts and surfaces with the alconox solution, followed by a tap water and a DI water rinse. Any metal or titanium surfaces must not come into contact with the 25% HNO3 solution as this may strip some metal alloys from these surfaces and introduce contamination.

10.2 Initial Calibration

Not applicable.

10.3 Equipment Operation and Sample Processing

10.3.1 Gloves must be worn when handling tissue samples.

10.3.2 Tissue samples should be partially thawed before starting, to the point where it becomes possible to make an incision in, or cut through, the flesh.

10.3.3 Note any morphological abnormalities on the processing records.

10.3.4 Fish Tissue Preparation

10.3.4.1 Determine the wet weight for each individual fish using a calibrated balance and appropriate weighing dish. Follow project specifications for alternate sample weight determinations.

10.3.4.2 Determine the length of each fish using a ruler, and record with the weight. Some measurements may, or may not be, a part of the project specifications. Additionally, a picture with a ruler in the foreground may be required. Follow project specifications.

10.3.4.3 Removal of Scales or Skin

10.3.4.3.1 If required by project specifications, the scales and/or skin of the fish will be removed prior to filleting. Clean all glassware and utensils as described in Section 10.1.

10.3.4.3.2 Rinse the fish with DI water and dry using a paper towel. Lay the fish on the cleaned, and/or lined, cutting board

Scrape the fish from tail to head using the blade edg**e** of a cleaned stainless steel, ceramic or titanium knife, to remove the scales. Continue until all scales are removed.

10.3.4.3.3 Depending upon the outward condition of the samples, the sample may be rinsed with Dl water and pat dry with paper towel. Place the fish on a clean cutting board, for filleting or skinning.

10.3.4.3.4 To skin the fish: Using a stainless steel knife, cut the skin behind the operculum (gill cover). Using the knife blade, pliers or other cleaned utensil, pull the skin off towards the tail of the fish. If necessary, cut lightly along the inside of the skin, slowly separating the skin from the muscle tissue. Removing the skin may require cutting the skin along the backbone or underbelly of the fish. If necessary follow project specifications for weight determinations.

10.3.4.4 Filleting the Fish

10.3.4.4.1 Using fresh gloves and the specified knife, make a cut behind the entire length of the operculum (gill cover), making sure to cut through the skin, if still attached, and the flesh, as close to the bone as possible. Note: If the fish samples are small, and it appears difficult to fillet, or if the amount of the fillet appears to be insufficient for the analysis requested, consult the Department Manager and/or Project Manager prior to filleting. In some cases it may be necessary to homogenize the whole body.

10.3.4.4.2 Make a cut across the caudal peduncle (the base of the tail fin) keeping as close to the caudal (tail) fin as possible. Continue cutting along the underbelly of the fish, moving from the head to the tail.

10.3.4.4.3 Go back to the cut made at the beginning at the operculum, and slice down the entire length of the fish following along the backbone until reaching the cut previously made across the caudal peduncle.

Gently slide the stainless knife along the backbone of the fish and along the rib cage. Remove the fillet from the fish. Be sure to include the belly flap in each fillet and do not remove the dark muscle tissue in the vicinity of the lateral line from the light muscle tissue that makes up the rest of the muscle tissue mass.

10.3.4.4.4 Remove any bones that may be left attached to the fillet. Repeat the fillet steps 10.3.4.4.1 through 10.3.4.4.3, for the opposite side of the specimen.

10.3.4.4.5 Note in the sample processing records if the internal organs were ruptured during freezing or if inadvertent puncture of the internal organs occurred during the filleting process. If the internal organs did rupture or were punctured, notify project manager for further guidance.

10.3.4.4.6 Place a glass plate on the balance. Tare the balance and record the appropriate weights in the appropriate spreadsheet or logbook as determined from the project specific QAPP. This may include weighing the fillet(s), carcass or skin.

10.3.4.4.7 If the fillet(s) and/or the carcass are to be homogenized immediately, proceed to Section 10.3.4.5. If not, store in the appropriate container; see Section 4 for allowable materials. Note that it may be necessary to chop the fillet(s) or carcass into smaller pieces, with the appropriately cleaned knife, before storage, and before homogenization, so the entire sample will fit into the storage container or the homogenization vessel. See the project specific QAPP for additional details.

10.3.4.4.8 If the samples will not be homogenized immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until homogenization

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10.3.4.5 Homogenization

10.3.4.5.1 Allow the fillet(s), carcass or whole body to partially thaw if previously frozen.

10.3.4.5.2 Fillets/Skin/Whole Body: Weigh a glass jar on the balance and record the weight. Tare the same glass jar. Be sure the jar is large enough to allow headspace for freezing after sample homogenization. While wearing the appropriate gloves, place the sample on the cutting board. Using the appropriate knife, slice and cut the sample into small chucks, preferably 1" squares or less. Add the sample to the appropriate size glass container for homogenization. Record the pre homogenization weight or follow project specific QAPP. Immerse the sample into the pre-cleaned generator probe (see section 10.1). Homogenize the sample until it appears fully and consistently homogenized tuning into a fine paste. This procedure may require mixing the sample during the homogenization process with a stainless steel spatula, ensuring all sample is equally processed and no sample remains on the side of the jar.

10.3.4.5.3 Large Whole Body/Carcass: Large sample carcasses may need to be homogenized using a hand held grinder/ electric grinder or food processor. Add the pre-sliced sample to the pre-cleaned blender (see section 10.1) and "push" through the auger part of the grinder. Collect the sample into a pre-tared jar or glass plate. Further processing using additional equipment may be necessary to achieve a consistently homogenized sample.

10.3.4.5.4 After homogenization, remove as much sample from the processing equipment as possible using a stainless steel spatula or other utensil and add to the processed sample. Re-weigh the sample and record the post-homogenization weight. Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior

to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.4.5.5 Place the individual or composite homogenized samples into the appropriate glass jars to be frozen pending future extraction/digestion. If the samples will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. All freezer logbooks must be filled out for hold time tracking purposes. Note the return of the samples to Sample Management must be documented in the LIMS Tracking log.

10.3.4.5.6 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1.

10.3.5 Mollusk Preparation

10.3.5.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes. Note removal of samples in the LIMS Tracking log.

10.3.5.2 If required by the project specifications, measure and record the length of the sample shell.

10.3.5.3 Cover the balance with the proper material as described in Section 4, and weigh and record the sample weight.

10.3.5.4 Wearing the proper gloves, place the sample on the cleaned cutting board. Samples should be partially thawed. If the sample is frozen, it will be difficult to break open the shell. If the sample is excessively thawed, the internal tissue will become soupy and difficult to remove.

10.3.5.5 If preparing *Bivalve* specimens, use the titanium knife to cut the abductor muscle by sliding the knife through the crevice where the two shells meet. Once the abductor muscle is cut the two shell pieces should come apart easily.

10.3.5.6 Carefully remove the top shell, and scoop out the internal tissue that is resting on the mantle. Be careful not to tip the bottom shell. If the sample is excessively thawed, the sample internal fluids may spill out of the shell. The internal fluids must be retained as part of the sample. If the bivalve is still partially frozen as suggested, the tissue should easily be removed from the shell in one piece.

10.3.5.7 Cover the balance with the proper material and weigh the amount of tissue obtained from the sample. Record the weight along with the information previously recorded on the processing records. The sample may now be stored pending homogenization in the appropriate jar, see Section 10.3.5.17. If the sample will be homogenized immediately, proceed to 10.3.5.13.

10.3.5.8 If preparing *Gastropod* specimens, a mallet will be necessary to open the shell.

10.3.5.9 Place a paper towel or piece of lab mat over the shell of the Gastropod specimen

10.3.5.10 Holding the shell still with one hand, use the mallet to hit the paper towel that is over the shell, in order to crush the shell.

10.3.5.11 Using the appropriately cleaned tweezers, remove the tissue from the crushed shell pieces.

10.3.5.12 Cover the balance with the proper material and weigh the amount of tissue obtained from the sample. Record the weight along with the information previously recorded on the processing records. The sample may now be stored pending homogenization in the appropriate jar, see Section 10.3.5.17. If the sample will be homogenized immediately, proceed to 10.3.5.13.

10.3.5.13 Since the amount of tissue obtained from one bivalve or gastropod is generally small, several specimens are frequently combined to make one sample. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be rinsed in between each composite sample.

10.3.5.14 If several specimens will be composited to make one sample, follow the applicable Sections of 10.3.5.1 through 10.3.5.11, for each of the specimens. The tissue obtained from each specimen may be weighed and recorded individually, then totaled for the composite weight. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight.

10.3.5.15 After the tissue has been removed from all of the specimen shells for one composite or individual sample, place the tissue in the clean small processor with the titanium blade to be homogenized. Grind the sample until it appears to be fully and consistently homogenized and there are no large chunks.

10.3.5.16 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.5.17 Place the processed samples into the appropriate glass jars to be frozen for future extraction/digestion, see Section 4. If the samples will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. Record placement of the samples in the freezer, in the freezer storage logbook, for hold time tracking. Note return of the samples to Sample Management in the LIMS Tracking log.

10.3.5.18 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1.

10.3.6 Crustacean Preparation

10.3.6.1 Lobsters

10.3.6.1.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes. Note removal of samples in the LIMS Tracking log.

10.3.6.1.2 If project specifications require gender determination of lobsters, this must be done prior to dissecting. To determine the gender, hold the lobster by the thorax, and flip it over to examine the underneath abdomen. Just below the legs and where the abdomen division begins, there is a first pair of swimmerets. The first pair of swimmerets is what is used to distinguish the lobster's gender. If the first pair is soft, has small hairs, and the swimmerets are crossed, it is **female**. On a **male** lobster, the first pair of swimmerets is hard and stiff, and generally do not touch.

10.3.6.1.3 If the hepatopancreas of the lobster samples is to be analyzed, the samples should be received alive. If the samples are frozen prior to dissection the hepatopancreas could burst upon thawing making it difficult to remove. To remove the hepatopancreas, the live lobster should be placed on a cleaned cutting board. Wearing the proper gloves, one analyst holds the two chelipeds (claws) out in front of the lobster, while also holding down the lower abdomen and telson (tail). The second analyst takes a knife, and places it on the grove in the carapace (outer shell), just behind the head region. Keeping the knife at an angle, the second analyst must push down and forward to remove the head. Once the head is removed the hepatopancreas can be seen lying just under the carapace and running the length of the thorax. The hepatopancreas is generally a greenish-yellow color, but there may be some variation. Scoop the hepatopancreas out gently trying not to break it into pieces. Cover the tray of the balance with the proper material, and weigh and record the weight of the hepatopancreas on the processing record, and place it into an appropriate sample jar for freezing and future extraction/ digestion.

10.3.6.1.4 To remove the edible meat, remove the two chelipeds from the body of the lobster at the joint. Place a piece of lab mat or paper towel over the cheliped and pound with a mallot. Once the shell is crushed remove the meat, using the appropriately cleaned tweezers or other tool, making sure to get all the meat in the joints and arms. Cover the balance tray with the appropriate material and weigh and record the total tissue weight obtained from the two chelipeds and arms. Record this weight with the previously recorded information on the sample processing record.

10.3.6.1.5 Remove the abdomen and telson from the rest of the carapace by pulling the lobster apart. Using the titanium coated knife, cut through

the center underside tissue of the lobster and laterally along the exoskeleton of the tail. Once the abdomen and tail have been cut open, separate the shell from the edible meat using cleaned utensils. Any eggs found in the female lobsters will have to be removed and discarded. Cover the balance tray with the appropriate material, and record the weight of the tissue obtained from the abdomen and telson on the processing record. The sample may now be stored pending homogenization in the appropriate jar.

10.3.6.2 Crabs

10.3.6.2.1 If removing tissue from *crabs* break off all legs and claws. Squeeze, pull, cut or pick all the tissue out of the legs and chelipeds. Pull apart the carapace. The carapace should be easy to remove by pulling up on the holes left from when the legs were broken off. Scoop out the tissue. Cover the balance tray with the appropriate material and record the weight of the tissue obtained from the legs, claws, and carapace on the processing record. The sample may now be stored pending homogenization in the appropriate jar, see Section 10.3.6.7. Any eggs found in the female crabs will have to be removed and discarded.

10.3.6.2.2 If the hepatopancreas of the crab samples is to be analyzed, the samples should be received alive. If the samples are frozen prior to dissection the hepatopancreas could burst upon thawing, making it difficult to remove. In order to remove the hepatopancreas of a frozen crab, remove the legs and claws, and then the top shell can be removed by cutting along the outside edge of the top shell. The top shell can then be removed. It is best if the crab(s) are chilled live in a refrigerator for 30-60 minutes, prior to removal of the hepatopancreas, to slow the crab's movements. To remove the hepatopancreas, the live crab should be placed on a cleaned cutting board. Wearing the proper gloves, the analyst must hold the crab still, with the claws facing away from the analyst. Then grab the back of the top shell with fingers or cleaned pliers, and pull the back shell from the crab. Once the back shell is removed the hepatopancreas can be seen lying inside the body cavity. The hepatopancreas is generally a greenish-yellow color, but there may be some variation. Scoop the hepatopancreas out gently trying not to break it into pieces. Cover the tray of the balance with the proper material, and weigh and record the weight of the hepatopancreas on the processing record. Place it into an appropriate sample jar for freezing and future extraction/ digestion.

10.3.6.3 Since the amount of tissue obtained from one crustacean may be small, several specimens may be combined to make one sample. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be cleaned and rinsed in between each composite sample.

10.3.6.4 If several specimens will be composited to make one sample, follow the applicable Sections of 10.3.6.1.1 through 10.3.6.1.5 for lobsters, or 10.3.6.2.1 through 10.3.6.2.2 for crabs for each of the specimens. The tissue obtained from each specimen may be weighed and recorded individually, then totaled for the composite weight. If only one composite

weight is sufficient for the project specifications, weigh the entire composite and record that weight.

10.3.6.5 After the tissue has been removed from all of the specimen shells for one composite or individual sample, grind the sample until it appears to be fully and consistently homogenized and there are no large chunks. This procedure may require mixing the sample during the homogenization process with a stainless steel spatula, ensuring all sample is equally processed and no sample remains on the side of the jar.

10.3.6.6 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.6.7 Place the processed samples into the appropriate glass jars to be frozen for future extraction/digestion, see Section 4 for allowable materials. If the samples will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. Record placement of the samples in the freezer, in the LIMS, for hold time tracking. Note return of the samples to Sample Management in the LIMS Tracking log.

10.3.6.8 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1. If any processing equipment comes in contact with a crab that is not going to be included in the composite, the equipment must be washed as described in section 10.1 before continuing.

10.3.7 Mammals (Mice and Shrew)

10.3.7.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes.

10.3.7.2 Place the first five, partially thawed samples to be processed, and all equipment needed into the glove box on a freshly laid out lab mat. Equipment needed includes:

- Empty and pre-labeled glass sample containers for the processed homogenate,
- PVC gloves or Latex gloves,
- 10% Bleach solution, 25% HNO3 and methylene chloride, methanol and hexane in squirt bottles,
- Omni grinding unit,
- Balance,
- Nylon bristled brushes,
- Ceramic, titanium,or stainless steel (organic compounds only) knives, spatulas and/or other utensils,
- Cutting board (s),

- Dl water in a squirt bottle and Kim wipes,
- Laboratory waste bottles with caps.

10.3.7.3 Once all materials are in the glove box and set up for use, seal the transfer box and ensure the motor blower is on. Over tightening of the outer or inner door knobs is not necessary to achieve a good seal. Place your hands into the gloves attached to the glove ports and place PVC or Latex gloves over the glove port gloves for use. The outer PVC or Latex gloves will need to be changed in between each sample.

10.3.7.4 If the gender of the mouse or shrew needs to be determined, turn the animal over and note the length of the anus and the distance of the anus from the tail. If the anus is elongated in shape and does not touch the base of the tail, testicles and a large genital papilla are visible, and there are no nipples, the animal is **male**. If the anus is round in shape and almost touches the base of the tail and/or there are nipples (up to five sets), the animal is **female**. If the animal is very small, young or immature and a gender determination cannot be made, note that the gender is *un-determined*. Record the gender observations on the processing records.

10.3.7.5 If skinning of the mammal is required, carefully make an incision at the tail end and cut just below the skin along the back, from one hind leg to the other. Make another cut from one hind leg to one front leg, and repeat the cut on the other side of the animal. Starting from the tail, lift the skin flap, and carefully separate the skin from the muscle tissue below. Pull the skin forward from the tail to the head to expose the back tissue of the animal. Repeat the procedure on the stomach side of the animal. Note: it may be very difficult to remove the skin from the legs, head and the tail. If some skin cannot be removed, note this on the processing records.

10.3.7.6 Weigh and record the weight of the mammal on the processing records. Depending upon the size of the mammal, it may need to be chopped into small pieces before being ground. Generally, mice and shrew can be quartered before homogenization if needed.

10.3.7.7 Put the whole body or chopped sample into the cup of the grinding unit. Ensure the sample is in contact with the blades of the unit and place a bag over the entire grinding unit to help contain and minimize splatter on the walls of the glove box.

10.3.7.8 Turn the grinding unit on low speed and gradually increase the speed to homogenize the sample being careful to minimize any splatter or outside contamination. Homogenize until a uniform consistency is achieved.

10.3.7.9 Transfer the homogenized sample from the cup to the pre-labeled sample jar using the appropriate utensil. Carefully clean the threads of the sample jar with a Dl water-soaked Kim wipe. Clean the outside of the sample jar with a 10% bleach-soaked Kim wipe. Set the sample jar inside the transfer box and close the transfer box inner door.

10.3.7.10 To clean the grinding unit in between samples, remove as much residual tissue on the blade as possible by operating the unit at low or medium speed with Dl water in the sample cup. Keep a bag over the grinding unit

as the primary containment for splashing. If necessary, use the nylon brush to gently scrub the exposed surfaces and to dislodge remaining tissue. Repeat as necessary, until the unit appears clean. Any plastic or ceramic parts must now be given a final rinse with 25% HNO3 then DI water when processing samples for metals analysis. If processing for organic compounds only, rinse with DI water, acetone and then the methylene chloride.

10.3.7.11 Repeat steps 10.3.2.3 through 10.3.7.10 until the five samples have been processed and each placed into the transfer box. Ensure the outer Latex or PVC gloves are changed in between each sample.

10.3.7.12 Since the amount of tissue obtained from one mouse or shrew may be small, several specimens may be combined to make one sample, as required by project specifications. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be cleaned in between each composite sample.

10.3.7.13 If several specimens will be composited to make one sample, follow the applicable Sections of 10.3.7.3 through 10.3.7.10, for each of the specimens. The tissue obtained from each specimen may be weighed and recorded individually, then totaled for the composite weight. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight.

10.3.7.14 Remove the individual or composite sample jars from the transfer box from the outside of the glove box, and return them to the Sample Management office for storage in the freezers until extraction/digestion. At the same time, obtain the next five samples to be processed and homogenized from the Sample Management office freezers. Movement of samples into and out of freezer storage must be documented in the freezer logbooks and in the LIMS Tracking log.

10.3.7.15 Allow the samples to partially thaw and begin again at 10.3.7.3 through 10.3.7.14 until all samples have been processed and homogenized. Clean the outer surfaces of the homogenate sample jars as described in 10.3.7.10, and remove them from the transfer box. If the samples will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. Record placement of the samples in the freezer, in the freezer storage logbook, for hold time tracking. Note return of the samples to Sample Management in the LIMS Tracking log.

10.3.7.16 Before removing any equipment from the glove box, the following disinfection steps must be taken:

- Remove the primary containment bag. Take care not to invert the bag. Place this bag into another bag.
- After the grinding unit, cup and blades have been cleaned with DI water as in 10.3.7.10, rinse the entire unit with the 10% bleach solution. Collect the bleach in a waste bottle.
- Remove the bags that were twist tie secured to the grinding unit, and place them into another bag. Rinse the entire unit again with the bleach solution.
- Roll up the bench liner, and place this into a bag.

- Pour all waste solutions into capped waste bottles. Place these bottles and any other bleach cleaned utensils, into bags, and seal all bags.
- Wipe the inside surfaces of the glove box with Kim wipes soaked in the bleach solution.
- The glove box transfer doors may now be opened to remove the grinding unit and waste. The waste material may be discarded after adding 10% bleach. The utensils and the grinding unit may be re-washed according to the normal cleaning procedures.

10.3.7.17 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.7.18 After individual homogenates have been combined to form the final sample composite homogenate, as requested, all utensils and equipment must be washed, in between samples, according to the procedures described previously in Section 10.1.

10.3.7.19 If the final sample composite homogenates will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. Record placement of the samples in the freezer, in the LIMS, for hold time tracking. Note return of the samples to Sample Management in the LIMS Tracking log.

10.3.8 Reptiles and Amphibians (Frogs and Turtles)

10.3.8.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes. Note removal of samples in the LIMS Tracking log.

10.3.8.2 Wearing the proper gloves, place the *turtle* sample on the cleaned cutting board. The turtle should be partially thawed. If the turtle is frozen, it will be difficult to remove the muscle. If the sample is excessively thawed, the internal tissue will become soupy and difficult to remove.

10.3.8.3 Take all project required measurements. The distance between the anterior and posterior edge of a turtle carapace (top of shell) should be measured with a ruler and recorded on the processing records. If the entire mass of the turtle, including the shell, needs to be recorded, cover the balance with the proper material and weigh and record this weight on the processing records.

10.3.8.4 Since the plastron (bottom of shell) and carapace are extremely dense and difficult to cut through with normal dissecting tools, the muscle tissue of the turtle must be removed by cutting the body of the turtle away from the

shell. Insert a knife, made of the proper material, into the skin of the turtle, close to the shell on the lower half of the body. Slowly, cut along the entire circumference of the shell. Repeat the procedure on the upper half of the body, on both sides of the shell.

10.3.8.5 With dissection scissors, or a ceramic or titanium paring knife of the proper material, remove the skin from the hind limbs, tail, fore limbs and neck.

10.3.8.6 Using the appropriate utensils, remove the muscle tissue from the tail, neck, hind limbs, and fore limbs, including the feet, leaving bone and claws behind. Remove any visible muscle tissue within the carapace. Most of this tissue will be found in the upper portion of the carapace around the pectoral area.

10.3.8.7 Cover the balance with the proper material and weigh the amount of tissue obtained from the turtle sample. Record the weight along with the information previously recorded on the processing records. The sample may now be stored pending homogenization in the appropriate jar, see Section10.3.8.15. If the sample will be homogenized immediately, proceed to 10.3.8.13.

10.3.8.8 If processing *frogs*, allow the frog to partially thaw, take the project specific measurements, and record them on the processing records. The number of frogs required to make up one sample, and the weight and length of the individual frogs, must be taken and recorded, if specified. In all cases, the skin must be removed from the frog prior to processing and chopped into smaller pieces, due to its thickness. It will then be added to the processor with the whole body of the frog, or it may be discarded depending upon the project specifications.

10.3.8.9 To skin the frog, make an incision, using the proper utensils, and cut into an area where there is an excess of skin, most likely around the neck. Slowly, pull the skin off of the frog using dissecting scissors, or a ceramic or titanium paring knife, as needed. Once skin is removed chop it up into tiny pieces using the appropriate knife and set it aside to be processed with the whole frog body.

10.3.8.10 Cover the balance with the proper material and weigh the amount of tissue obtained from the frog sample, if the tissue and not the whole body will be processed. Record the weight along with the information previously recorded on the processing records. The sample may now be stored pending homogenization in the appropriate jar, see Section 10.3.8.15. If the sample will be homogenized immediately, proceed to 10.3.8.13.

10.3.8.11 Since the amount of tissue obtained from one small turtle or frog may be insignificant, several specimens may be combined to make up one sample. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be rinsed in between each composite sample.

10.3.8.12 If several specimens will be composited to make up one sample, follow the applicable Sections of 10.3.8.1 through 10.3.8.10, for each of the specimens. The tissue obtained from each specimen may be weighed and recorded individually, then totaled for the composite weight. If only the

composite weight is sufficient for the project specifications, weight the entire composite and record that weight.

10.3.8.13 After the tissue has been removed from all of the specimens, homogenize the muscle tissue, and skin if required, by placing it into the small or large food processor fitted with the appropriate blades (stainless steel for the large processor and titanium for the small processor). See Section 4 for allowable materials. The sample may need to be cut into smaller pieces for processing. Grind the sample until it appears to be fully and consistently homogenous. Continue to grind the sample until there are no chunks present in the homogenate.

10.3.8.14 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.8.15 Individual or composite samples may be returned to the Sample Management office for further storage in freezers pending extraction/digestion. All processed samples are stored in the proper containers noted in Section 4. All freezer logbooks must be filled out for hold time tracking purposes. Return of samples to Sample Management must be documented in the LIMS Tracking log.

10.3.8.16 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1.

10.3.9 Macro Invertebrates

10.3.9.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes. Note removal of samples in the LIMS Tracking log.

10.3.9.2 Cover the balance tray with the appropriate material and record the weight of the invertebrate sample. Since the weight obtained from one invertebrate (benthic worms, insects or biota) may be small, several invertebrates may be combined to make one sample. In many cases, several invertebrates of the same species and sample location are delivered to the laboratory in one sample jar. Each specimen from this jar must be weighed, if requested, and composited to form one homogenized and unique sample. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight. Utensils do not need to be rinsed between the individual samples or specimens that comprise one composite, but utensils must always be rinsed in between each composite sample.

10.3.9.3 Invertebrates such as *eels* must be chopped into smaller pieces before homogenization. This is generally due to the length of the specimen and the thickness of the skin.

10.3.9.3.1 For project specifications requiring eel specimens to be skinned prior to homogenization, first secure eel to cutting board using a stainless steel screw. Using a stainless steel knife, cut the skin behind the operculum (gill cover). Using the knife blade, pliers or other cleaned utensil, pull the skin off towards the tail. If necessary, cut lightly along the inside of the skin, slowly separating the skin from the muscle tissue. Removing the skin may require cutting the skin along the backbone or underbelly. If necessary follow project specifications for weight determinations.

10.3.9.4 Place the weighed specimen into the clean small processor with the titanium blade to be homogenized. Process the sample until it appears to be fully and consistently homogenized and there are no large chunks.

10.3.9.5 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.9.6 Individual or composite samples may be returned to the Sample Management office for further storage in freezers pending extraction/digestion. All homogenates are stored in the proper containers noted in Section 4. All freezer logbooks must be filled out for hold time tracking purposes. Return of samples to Sample Management must be documented in the LIMS Tracking log.

10.3.9.7 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1.

10.3.10 Plants

10.3.10.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 10.1. Note the allowable materials in Section 4. Obtain samples from the Sample Management office and log them out of the freezer logbooks for hold time tracking purposes. Note removal of samples in the LIMS Tracking log.

10.3.10.2 Wearing the appropriate gloves, plants must be rinsed with Dl water to remove soil, silt, small insects, and other debris. Place the plants in a stainless steel or plastic strainer, depending on the determinative sample analysis, and rinse thoroughly with Dl water. If analyzing the sample for metals and organic compounds, rinse the plants carefully over a sink, being sure not to touch the sides of the sink with the plant sample.

10.3.10.3 Depending on the size and texture of the plants, some may be homogenized in the small food processor with the titanium blade. Samples such as long grass will have to be chopped into small pieces (approximately 1/2 inch) using titanium or ceramic knives. Leaves can generally be homogenized in the small food processor without pre-cutting.

10.3.10.4 Some project specifications may require the plants to be dried prior to homogenization. A plastic salad spinner may be used to remove excess water from samples, if organic compounds do not need to be determined. If both metals and organic compounds need to be determined, air drying for 48 hours, or oven drying overnight at low temperatures (S 50°C), can be done. Freeze drying the plant is an additional option for the removal of water and may be employed per project specifications.

10.3.10.5 Cover the balance tray with the appropriate material and record the weight of the plant sample. Since the weight obtained from one plant may be small, several plants may be combined to make one sample. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be rinsed in between each composite sample.

10.3.10.6 If several plants will be composited to make one sample, follow the applicable Sections of 10.3.10.2 through 10.3.10.5, for each of the specimens. The weight of each specimen may be recorded individually, and then totaled for the composite weight. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight on the processing records.

10.3.10.7 After the plant weight for one composite or individual sample has been recorded, place the plant(s) in the clean small processor with the titanium blade to be homogenized, or place them onto the cleaned cutting board to be chopped. Grind or chop the plants until they appear to be fully homogenized.

10.3.10.8 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

10.3.10.9 Place the homogenized plants into the appropriate glass jars to be frozen for future extraction/digestion, see Section 4. If the samples will not be extracted/digested immediately, the samples must be returned to the Sample Management office and placed back into the freezer, until extraction/digestion. Record placement of the samples in the freezer, in the freezer storage logbook, for hold time tracking. Return of samples to Sample Management must be documented in the LIMS Tracking log.

10.3.10.10 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 10.1.

10.4 Continuing Calibration

Not applicable.

10.5 Preventive Maintenance

Not applicable to this method.

11. Data Evaluation, Calculations and Reporting

The processing bench sheets and other relevant laboratory notebooks must follow the specifications in the ALPHA ANALYTICAL *Logbook Usage Work Instructions* (WI 108-01), and all record keeping and document control practices. Separate project-specific documents may be used in place of Alpha bench sheets, as necessary.

See the appropriate ALPHA analytical SOPs noted in Section 1, for details on sample analysis, data evaluation, calculations and data reporting.

All results for the organic/inorganic compounds of interests are reportable without qualification if extraction/digestion and analytical holding times are met, preservation (including cooler and freezer temperatures) are met, all QC criteria defined in the table below are met, and matrix interference is not suspected during extraction/digestion and/or analysis of the samples. If any of the below QC parameters are not met, all associated samples must be evaluated for re-extraction and/or re-analysis.

QC Parameter	Acceptance Criteria
Equipment/Processing Blank	< reporting limit
Method Blank	< reporting limit
Laboratory Control Sample	See the applicable ALPHA analytical SOP for acceptance criteria
Matrix Duplicate	See the applicable ALPHA analytical SOP for acceptance criteria
Matrix Spike	See the applicable ALPHA analytical SOP for acceptance criteria
Matrix Spike Duplicate	See the applicable ALPHA analytical SOP for acceptance criteria
Surrogate Recoveries	See the applicable ALPHA analytical SOP for acceptance criteria
Standard Reference Material	See the applicable ALPHA analytical SOP for acceptance criteria

12. Contingencies for Handling Out-of-Control Data or Unacceptable Data

Section 9, Quality Control, defines the preparation and/or analytical corrective actions that must be taken in instances where QC outliers exist.

Section 11 outlines sample batch QC acceptance criteria. If non-compliant organic or inorganic compound analytical results are to be reported, the Department Manager and/or the Laboratory Director, and the QA Manager must approve the reporting of these results. The laboratory Project Manager shall be notified, and may chose to relay the non-compliance to the client, for approval, or other corrective action, such as re-sampling and re-analysis. The analyst or Department Manager performing the secondary review initiates the project narrative, and the narrative must clearly document the non-compliance and provide a reason for acceptance of these results.

13. Method Performance

13.1 Method Detection Limit Study (MDL) / Limit of Detection Study (LOD) / Limit of Quantitation (LOQ) – Not Applicable

Not Applicable

13.2 Demonstration of Capability Studies

Not Applicable

14. Pollution Prevention and Waste Management

Refer to Alpha's Chemical Hygiene Plan and Hazardous Waste and Sample Disposal SOP for further pollution prevention and waste management information.

Once satisfactory organic or inorganic compound results have been generated, the extracts/digestates are held for 30 days, or longer, if specified by a client contract. Then, organic extracts are discarded into a 55-gallon drum labeled "Vial Waste" and inorganic digestates are poured into a 55-gallon drum marked "Acid/Non-chlorinated" waste.

All solvent or reagent waste generated during processing and/or extraction/digestion must be stored in satellite containers in the preparation laboratories labeled "Organic Solvent", "Acid/Non-chlorinated" or "Bleach".

Once the organic solvent satellite containers are full, they must be emptied into 55-gallon drums marked "Organic Solvent Waste". Cleanup waste from the HPLC fractionators (silica cleanup) or GPC is emptied into the 55-gallon drum marked "HPLC Solvent Waste". Bleach from disinfection is emptied into the 20-gallon drum marked "Bleach", and reagent waste generated during metals analysis is emptied into a 55-gallon drum marked "Acid/Non-chlorinated" waste.

15. Referenced Documents

Chemical Hygiene Plan

SOP/08-05 MDL/LOD/LOQ Generation

SOP/08-12 IDC/DOC Generation

SOP/G-006 Hazardous Waste and Sample Disposal

16. Attachments

None.

Attachment X: SOP—Analysis of Parent and Alkylated Polynuclear Aromatic Hydrocarbons, Selected Heterocyclic Compounds, Steranes, Triterpanes, and Triaromatic Steroids by GC/MS – SIM

SOP is included in folio as separate PDF file.

Attachment Y: SOP—Alumina Column Cleanup of Organic Extracts

SOP is included in folio as separate PDF file.

Attachment Z: Caged Bivalve Pilot Study Results Memorandum

200 West Mercer St. • Suite 401 • Seattle, WA 98119
Phone: 206.378.1364 • Fax: 206.217.0089 • www.windwardenv.com

MEMORANDUM

To:	Cooperating Parties Group
From:	Thai Do, Karen Tobiason, and Lisa Saban, Windward Environmental LLC
Subject:	Caged Bivalve Pilot Study Results
Date:	January 20, 2011

This memorandum provides a summary of the results and observations recorded during the 45-day caged bivalve pilot study requested by the US Environmental Protection Agency (USEPA) and conducted by the Cooperating Parties Group (CPG) in the Lower Passaic River Study Area (LPRSA). As specified in the USEPA-approved study design for the caged bivalve pilot study (Windward 2010), the purpose of the pilot study was to determine if the Eastern oyster (*Crassostrea virginica*) would survive in the estuarine portion of the LPRSA and, further, to determine whether the oyster or the ribbed mussel (*Geukensia demissa*) would be more suitable for use in the estuarine zone during a 90-day caged bivalve study anticipated to begin in spring 2011.

Two locations were selected for the side-by-side pilot study based on target salinity ranges agreed to by USEPA and CPG (i.e., 6 to 15 parts per thousand [ppt] for one location and 2 to 3 ppt for the other). LPR-CBPS1, located at River Mile (RM) 3.9 on the east side of the river, had a target salinity range of 6 to 15 ppt; and LPR-CBPS2, located at RM 6.8 on the west side of the river, had a target salinity range of 2 to 3 ppt. Test cage deployment and retrieval locations are shown on Figure 1.

The pilot study was conducted from November 11, 2010 (Day 0), through January 6, 2011 (Day 45). Test cages were deployed at the two selected locations on Day 0, checked midway through the study (Day 22), and retrieved at test termination (Day 45). On Days 0, 22, and 45, temperature and salinity measurements were recorded at 1-ft intervals in the water column at each location, and all oysters and mussels were examined externally for survival at Days 22 and 45. In addition, individual bivalves were sacrificed on Day 0 (ten each of oysters and mussels) and Day 22 (two from each cage) to conduct an internal inspection. The inspections documented tissue tone (e.g., firm or loose), general color (e.g., milky vs. clear and watery), any odors (e.g., rancid smell), and mantle appearance (e.g., color, transparency). On Day 45, all individuals

remaining at the end of the test period were internally inspected. Field-collected data, including individual length measurements, water salinity and temperature measurements, and records from the internal inspections are provided in Appendix A. Photos are provided in Appendix B.

BIVALVE PROCUREMENT

Oysters and mussels used in the caged bivalve pilot study were obtained from Aquatic Research Organisms, Hampton, New Hampshire. The bivalves were field-collected from Cape Cod estuaries on November 16, 2010, divided into two batches, and acclimated over a period of 3 days to target salinity range and water temperature. One batch was acclimated to a salinity of 10 ppt, and the other was acclimated to a salinity of 2 ppt. Both batches were acclimated to the water temperatures at the two test locations (i.e., approximately 11 ˚C). Organisms were held at the target salinity for 3 days prior to deployment.

DAY 0

Prior to test cage deployment on November 22, 2010, all oysters and mussels were measured for length (see Table 1 in Appendix A), and five individuals from each batch of bivalves (i.e., oysters acclimated to salinities of 2 or 10 ppt; mussels acclimated to salinities of 2 or 10 ppt) were sacrificed for an internal inspection. The inspections were conducted to examine the tissue for tone, general color, odor, mantle appearance, and the presence of parasites. The tissue from the 20 sacrificed bivalves (e.g., 10 mussels and 10 oysters) appeared to be normal based on the criteria used for internal tissue examination (Appendix A).

Side-by-side cages, each containing either 30 mussels or 30 oysters were deployed at each of the two locations. Cages deployed at LPR-CBPS1 and LPR-CBPS2 contained bivalves acclimated to a salinity of 10 ppt and 2 ppt, respectively. Bivalves were first placed into a mesh bag and zip-tied into individual compartments (see Photos 4 through 6 in Appendix B) and then placed in rigid cages (with an attached surface buoy) mounted on cement blocks to hold the cages 1 ft off the bottom of the river (see Photo 7 in Appendix B).

Cages were deployed during low tide to ensure that they would remain completely submerged throughout the test period. Salinity and temperature measurements were taken at 1-ft intervals at both locations. The salinity and temperature measurements taken at three depths at LPR-CBPS1 were the same (i.e., a salinity of 4.95 ppt and temperature of 10.52°C).

At LPR-CBPS2, the salinity and temperature measurements were 0.42 ppt and 9.82°C, respectively. The salinity was lower than the targeted salinity range (2 to 3 ppt) for that location. After consultation with USEPA, it was agreed that because deployment occurred at low tide when the salinity in this portion of the river is lowest, the cages would be left at the proposed location until salinity could be rechecked the following

day during high tide. If the salinity was still not within the targeted range, the test cages would be relocated to a location that met the salinity range requirement.

DAY 1

On Day 1, the salinity at both locations was rechecked at cage depth (i.e., 1 ft off the bottom of the river). The salinity at LPR-CBPS1 was 9.67 ppt, which was within the target salinity range of 6 to 15 ppt for that location. Therefore, the cages at LPR-CBPS1 were left in place. The salinity at LPR-CBPS2 was 1.54 ppt, which was below the target salinity of 2 to 3 ppt. The test cages at LPR-CBPS2 were relocated downriver to a new location at RM 6.3, where the salinity and temperature were recorded at 2.3 ppt and 10.5°C, respectively, as measured 1 ft off the bottom of the river.

DAY 22

On December 14, 2010, the test cages were retrieved (during the low-tide cycle), and all bivalves were checked for survival. Water temperature and salinity measurements were taken at both locations. Two bivalves from each test cage were sacrificed and the tissue was inspected for tone, general color, odor, mantle appearance, and the presence of parasites. All mussels and oysters at both locations were tightly closed, and all were considered to be alive (i.e., 100% survival). Tissue from the sacrificed bivalves appeared to be normal upon examination based on the criteria used for internal tissue examination. The four cages, each containing 28 organisms (either mussels or oysters), were returned to the river.

At LPR-CBPS1, the salinity measured at 1 ft above the river bottom was 11.47 ppt, which was within the target salinity range for this location (i.e., 6 to 15 ppt). The water temperature measured at 1 ft above the river bottom was 5.97°C.

At LPR-CBPS2, the salinity and temperature measured at 1 ft above the river bottom were 0.30 ppt and 4.09°C, respectively. The salinity measured at LPR-CBPS2 was below the target salinity range of 2 to 3 ppt, but the reading was recorded at low tide when salinity readings were at their lowest as a result of freshwater influence. As noted in the USEPA-approved memorandum describing the design for the caged bivalve pilot study (Windward 2010), the salinity at any location in the river will fluctuate based on tidal influence and the volume of fresh water discharging into the system.

DAY 45

On January 6, 2011, all four test cages were retrieved (during the high-tide cycle), and bivalves were checked for survival. All mussels at both locations and all oysters at LPR-CBPS1 survived (e.g., 100% survival). At LPR-CBPS2, 39% (11 out of 28) of the oysters survived. The tissue of all surviving mussels and oysters was examined and found to be alive based on the criteria used for internal tissue examination. An internal inspection of the dead oysters was also conducted (see Photos 26 and 27). During

inspection of the dead oysters, sediment was found inside the shells, and at least three of the oysters were detached from their shells.

During retrieval, it was observed that five mussels from LPR-CBPS2 were gaping and did not immediately respond (i.e., close) when tapped. However, during the examination, the five mussels had closed, and an inspection of the tissue showed that they were still alive. Gaping is not atypical behavior for mussels, and the cold temperatures may have contributed to their slow response. During examination upon the retrieval of the cages, it was noted that the valves (i.e., shells) of many of the mussels appeared to be worn. However, upon comparison of the mussel photos taken on Day 45 with those taken on Day 0 (prior to deployment at test initiation), no clear difference between the in the amount of wear condition of the valves was detected. Photo 4 shows the wear on the mussel valves on Day 0; and Photos 13, 19, and 20 show the wear on the mussel valves on Day 45 (see Appendix B).

At LPR-CBPS1, the salinity measured at 1 ft above the river bottom was 14.85 ppt, which was within the target range of 6 to 15 ppt. The water temperature measured at 1 ft above the bottom of the river was 2.74°C.

At test termination, neither of the cages deployed at LPR-CBPS2 was located at the deployment site. Both cages had been dragged from the deployment location sometime between Day 22 and Day 45, presumably by debris in the river. The test cage that contained the oysters was carried downriver approximately 1,000 ft (referred to as LPR-CBPS2a), where the salinity measured at 1 ft above the river bottom was 5.98 ppt, and the temperature was 2.04°C. The test cage that contained the mussels was carried upriver approximately 2,500 ft (referred to as LPR-CBPS2b), where the salinity measured at 1 ft above the river bottom was 4.36 ppt, and the temperature was 2.01°C.

RECOMMENDATIONS

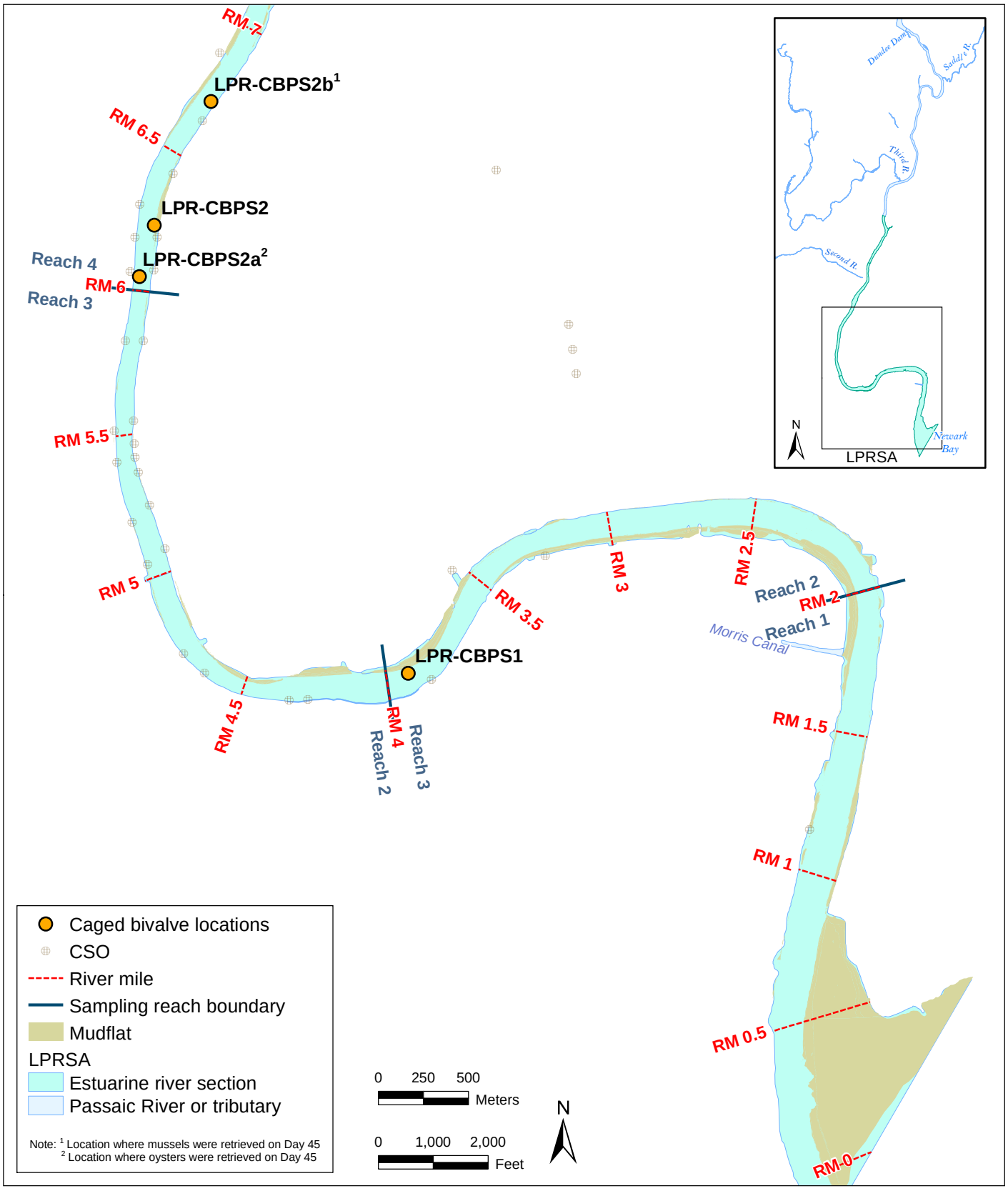
Results from the caged bivalve pilot study indicate that both Eastern oysters and ribbed mussels survived at the test location in the lower portion of the estuarine zone (RM 3.9). At the end of the 45-day deployment, 100% of both the mussels and oysters had survived at that location, which targeted a salinity range of 6 to 15 ppt (LPR-CBPS1), and the results of the internal examinations of individuals at Day 45 were similar to those of individuals examined at Days 0 and 22. However, at the low-salinity location farther up the river (LPR-CBPS2, at RM 6.3), significant differences in survival between the mussels and oysters were observed. At the end of the 45-day deployment, oyster survival was 39% as compared with 100% survival for the mussels. These results indicate that the oysters are not a suitable test species for the low-salinity conditions of the upper portion of the estuarine zone.

Based on the results from the 45-day caged bivalve pilot study, Windward concludes that the ribbed mussel is the appropriate test species for the entire estuarine portion of the LPRSA for the 90-day caged bivalve study because it is the species that had the

higher survivorship and appears to be better able to tolerate the wide-ranging salinity conditions encountered in the estuarine portion of the LPRSA.

REFERENCES

Windward. 2010. Memorandum dated November 16, 2010, to de maximis, inc.: Caged bivalve study. Lower Passaic River Restoration Project. Lower Passaic River Study Area RI/FS. Windward Environmental LLC, Seattle, WA.



Mudflats and gravel flats are areas where the river bottom slope is less than or equal to 6°and the depth is >-4.5 feet NGVD29 (i.e.>-2 feet MLLW). Flats along the navigation channel were determined based on the 2007 bathymetry data from Rogers Surveying (for USACE). The Kearney Point mudflat is outside the survey area and was estimated from NOAA bathymetry.

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Prepared at Request of Counsel

Figure 1. Caged bivalve pilot study locations

DRAFT

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Appendix A Field-collected Data

Table 1. Bivalve lengths at test initiation

Parameter	LPR-CBPS1		LPR-CBPS2	
	Mussels	Oysters	Mussels	Oysters
Minimum length (mm)	48	60	51	56
Maximum length (mm)	62	73	65	71
Mean length (mm)	55.4	67.1	55.8	66.2
Number of organisms	30	30	30	30

Table 2. Water quality measurements

Depth (ft)	Salinity (ppt)	Temperature (°C)	Depth (ft)	Salinity (ppt)	Temperature (°C)
Day 0					
LPR-CBPS1					
1	4.95	10.52	3	4.95	10.52
2	4.95	10.52			
LPR-CBPS2					
na[a]	2.30	10.50			
Day 22					
LPR-CBPS1					
1	0.81	4.77	7	3.07	5.12
2	0.81	4.78	8	7.94	5.28
3	0.81	4.79	9	9.67	5.69
4	0.86	4.78	10	10.70	5.85
5	1.04	4.83	11	11.06	5.90
6	2.50	4.99	12	11.47	5.97
LPR-CBPS2					
1	0.17	4.04	4	0.19	4.07
2	0.17	4.06	5	0.21	4.08
3	0.17	4.06	6	0.30	4.09

Table 2. Water quality measurements

Depth (ft)	Salinity (ppt)	Temperature (°C)	Depth (ft)	Salinity (ppt)	Temperature (°C)
Day 45					
LPR-CBPS1					
1	8.25	2.35	8	13.80	2.59
2	8.44	2.26	9	14.45	2.61
3	11.29	2.43	10	14.52	2.66
4	12.35	2.48	11	14.51	2.68
5	13.09	2.55	12	14.77	2.69
6	13.22	2.58	13	15.05	2.72
7	13.56	2.58	14	14.85	2.74
LPR-CBPS2a					
1	4.56	1.92	6	5.53	2.00
2	4.71	1.94	7	5.57	2.01
3	4.85	1.96	8	5.75	2.02
4	5.02	1.97	9	5.88	2.03
5	5.32	1.99	10	5.98	2.04
LPR-CBPS2b					
1	2.06	1.75	11	4.33	1.98
2	3.07	1.82	12	4.35	1.99
3	3.19	1.84	13	4.40	1.99
4	3.40	1.87	14	4.44	1.99
5	3.54	1.88	15	4.36	2.00
6	3.91	1.90	16	4.31	2.00
7	3.97	1.94	17	4.34	2.00
8	3.99	1.95	18	4.35	2.00
9	4.16	1.96	19	4.36	2.01
10	4.29	1.97			

[a] Salinity and temperature measurements were taken on Day 1 after the test cages had been moved to a location farther downriver that met the target salinity requirements of 2 to 3 ppt (LPR-CBPS2). Measurements taken at the initial deployment location on Day 0 are not included in this table. A depth measurement is not available because the subcontractor did not record the depth when the cages were moved.

C – Celsius

ppt – parts per thousand

Table 3. Bivalve internal exam data

Species	Length (mm)	Width (mm)	Depth (mm)	Gape	Mantle	Color	Tone	Odor	Parasites	Comments
Day 0										
LPR-CBPS1										
Mussel	58	25	15	closed	thick	pale yellow	firm	none	none	alive
	54	21	15	closed	thick	pale yellow	firm	none	none	alive
	57	24	16	closed	thick	pale yellow	firm	none	none	alive
	59	23	15	closed	thick	pale yellow	firm	none	none	alive
	52	22	13	closed	thick	pale yellow	firm	none	none	alive
Oyster	68	42	14	closed	thick	white	firm	none	none	alive
	58	44	20	closed	thick	white	firm	none	none	alive
	72	49	22	closed	thick	white	firm	none	none	alive
	60	50	11	closed	thick	white/pale yellow	firm	none	none	alive
	74	46	17	closed	thick	white/pale yellow	firm	none	none	alive
LPR-CBPS2										
Mussel	58	26	16	closed	thick	pale yellow	firm	none	none	alive
	60	58	18	closed	thick	pale yellow	firm	none	none	alive
	61	23	15	closed	thick	pale yellow	firm	none	none	alive
	57	24	17	closed	thick	pale yellow	firm	none	none	alive
	50	21	13	closed	thick	pale yellow	firm	none	none	alive
Oyster	60	41	15	closed	thick	white/pale yellow	firm	none	none	alive
	72	46	21	closed	thick	off-white	firm	none	none	alive
	64	50	20	closed	thick	off-white	firm	none	none	alive
	68	55	13	closed	thick	off-white	firm	none	none	alive
	65	49	15	closed	thick	off-white	firm	none	none	alive
Day 22										
LPR-CBPS1										
Mussel	59	25	16	closed	thick	yellow	firm	none	none	alive
	59	23	15	closed	thick	yellow	firm	none	none	alive
Oyster	67	45	22	closed	thick	off-white	firm	none	none	alive
	66	45	18	closed	thick	off-white	firm	none	none	alive
LPR-CBPS2										
Mussel	63	25	16	closed	thick	yellow	firm	none	none	alive
	58	25	14	closed	thick	yellow	firm	none	none	alive
Oyster	64	42	18	closed	thick	light gray	firm	none	none	alive
	71	49	19	closed	thick	pale gray	firm	none	none	alive

Table 3. Bivalve internal exam data

Species	Length (mm)	Width (mm)	Depth (mm)	Gape	Mantle	Color	Tone	Odor	Parasites	Comments
Day 45										
LPR-CBPS1										
Mussel	55	21	14	closed	thick	pale yellow	firm	none	none	alive
	51	20	13	closed	thick	pale yellow	firm	none	none	alive
	55	21	14	closed	thick	pale yellow	firm	none	none	alive
	56	21	14	closed	thick	pale yellow	firm	none	none	alive
	56	22	15	closed	thick	pale yellow	firm	none	none	alive
	56	23	14	closed	thick	pale yellow	firm	none	none	alive
	58	22	13	closed	thick	pale yellow	firm	none	none	alive
	58	24	16	closed	thick	pale yellow	firm	none	none	alive
	52	22	13	closed	thick	pale yellow	firm	none	none	alive
	55	22	15	closed	thick	pale yellow	firm	none	none	alive
	65	24	17	closed	thick	pale yellow	firm	none	none	alive
	61	25	17	closed	thick	pale yellow	firm	none	none	alive
	50	20	12	closed	thick	pale yellow	firm	none	none	alive
	58	24	15	closed	thick	pale yellow	firm	none	none	alive
	59	23	14	closed	thick	pale yellow	firm	none	none	alive
	55	24	13	closed	thick	pale yellow	firm	none	none	alive
	54	21	14	closed	thick	pale yellow	firm	none	none	alive
	56	22	14	closed	thick	pale yellow	firm	none	none	alive
	54	21	15	closed	thick	pale yellow	firm	none	none	alive
	53	21	15	closed	thick	pale yellow	firm	none	none	alive
	58	25	14	closed	thick	pale yellow	firm	none	none	alive
	55	23	15	closed	thick	pale yellow	firm	none	none	alive
	51	24	14	closed	thick	pale yellow	firm	none	none	alive
	50	21	13	closed	thick	pale yellow	firm	none	none	alive
	57	20	15	closed	thick	pale yellow	firm	none	none	alive
	55	22	13	closed	thick	pale yellow	firm	none	none	alive
	53	22	15	closed	thick	pale yellow	firm	none	none	alive
	59	22	14	closed	thick	pale yellow	firm	none	none	alive

Table 3. Bivalve internal exam data

Species	Length (mm)	Width (mm)	Depth (mm)	Gape	Mantle	Color	Tone	Odor	Parasites	Comments
Oyster	63	53	21	closed	thick	pale white/gray	firm	none	none	alive
	66	48	21	closed	thick	pale white/gray	firm	none	none	alive
	66	44	16	closed	thick	pale white/gray	firm	none	none	alive
	64	48	18	closed	thick	pale white/gray	firm	none	none	alive
	69	45	20	closed	thick	pale white/gray	firm	none	none	alive
	67	40	18	closed	thick	pale white/gray	firm	none	none	alive
	70	44	17	closed	thick	pale white/gray	firm	none	none	alive
	66	47	17	closed	thick	pale white/gray	firm	none	none	alive
	61	40	15	closed	thick	pale white/gray	firm	none	none	alive
	70	43	20	closed	thick	pale white/gray	firm	none	none	alive
	70	37	15	closed	thick	pale white/gray	firm	none	none	alive
	64	44	21	closed	thick	pale white/gray	firm	none	none	alive
	65	44	19	closed	thick	pale white/gray	firm	none	none	alive
	55	41	13	closed	thick	pale white/gray	firm	none	none	alive
	59	43	17	closed	thick	pale white/gray	firm	none	none	alive
	63	47	17	closed	thick	pale white/gray	firm	none	none	alive
	69	42	18	closed	thick	pale white/gray	firm	none	none	alive
	66	52	17	closed	thick	pale white/gray	firm	none	none	alive
	65	43	17	closed	thick	pale white/gray	firm	none	none	alive
	58	43	18	closed	thick	pale white/gray	firm	none	none	alive
	68	49	21	closed	thick	pale white/gray	firm	none	none	alive
	70	49	20	closed	thick	pale white/gray	firm	none	none	alive
	60	47	17	closed	thick	pale white/gray	firm	none	none	alive
	67	49	20	closed	thick	pale white/gray	firm	none	none	alive
	69	55	20	closed	thick	pale white/gray	firm	none	none	alive
	69	40	18	closed	thick	pale white/gray	firm	none	none	alive
	71	49	21	closed	thick	pale white/gray	firm	none	none	alive
	68	50	16	closed	thick	pale white/gray	firm	none	none	alive

Table 3. Bivalve internal exam data

Species	Length (mm)	Width (mm)	Depth (mm)	Gape	Mantle	Color	Tone	Odor	Parasites	Comments
LPR-CBPS2										
Mussel	58	23	15	closed	thick	pale yellow	firm	none	none	alive
	58	23	15	closed	thick	pale yellow	firm	none	none	alive
	58	24	15	closed	thick	pale yellow	firm	none	none	alive
	55	21	14	closed	thick	pale yellow	firm	none	none	alive
	55	22	13	closed	thick	pale yellow	firm	none	none	alive
	51	20	15	closed	thick	pale yellow	firm	none	none	alive
	58	23	15	closed	thick	pale yellow	firm	none	none	alive
	56	22	14	closed	thick	pale yellow	firm	none	none	alive
	62	25	16	closed	thick	pale yellow	firm	none	none	alive
	60	23	14	closed	thick	pale yellow	firm	none	none	alive
	52	21	15	closed	thick	pale yellow	firm	none	none	alive
	52	20	13	closed	thick	pale yellow	firm	none	none	alive
	47	19	12	closed	thick	pale yellow	firm	none	none	alive
	55	23	14	closed	thick	pale yellow	firm	none	none	alive
	56	22	16	closed	thick	pale yellow	firm	none	none	alive
	55	21	14	closed	thick	pale yellow	firm	none	none	alive
	61	25	14	closed	thick	pale yellow	firm	none	none	alive
	55	21	13	closed	thick	pale yellow	firm	none	none	alive
	52	22	13	closed	thick	pale yellow	firm	none	none	alive
	57	22	14	closed	thick	pale yellow	firm	none	none	alive
	49	20	14	closed	thick	pale yellow	firm	none	none	alive
	51	22	12	closed	thick	pale yellow	firm	none	none	alive
	56	22	15	closed	thick	pale yellow	firm	none	none	alive
	60	21	14	slightly ajar	thick	pale yellow	firm	none	none	alive
	58	24	16	slightly ajar	thick	pale yellow	firm	none	none	alive
	52	20	13	slightly ajar	thick	pale yellow	firm	none	none	alive
	55	24	14	slightly ajar	thick	pale yellow	firm	none	none	alive
	52	21	14	slightly ajar	thick	pale yellow	firm	none	none	alive

Table 3. Bivalve internal exam data

Species	Length (mm)	Width (mm)	Depth (mm)	Gape	Mantle	Color	Tone	Odor	Parasites	Comments
Oyster	70	42	17	closed	thick	pale white/gray	firm	none	none	alive
	68	39	14	closed	thick	pale white/gray	firm	none	none	alive
	69	44	21	closed	thick	pale white/gray	firm	none	none	alive
	64	47	20	closed	thick	pale white/gray	firm	none	none	alive
	61	38	18	closed	thick	pale white/gray	firm	none	none	alive
	66	44	23	closed	thick	pale white/gray	firm	none	none	alive
	68	49	20	closed	thick	pale white/gray	firm	none	none	alive
	65	39	17	closed	thick	pale white/gray	firm	none	none	alive
	71	42	17	closed	thick	pale white/gray	firm	none	none	alive
	64	45	19	closed	thick	pale white/gray	firm	none	none	alive
	64	51	17	closed	thick	pale white/gray	firm	none	none	alive
	65	42	20	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	62	41	17	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	62	54	20	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	72	40	20	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	71	41	17	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	65	41	18	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	72	47	21	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	73	46	17	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	65	43	19	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	67	45	17	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	71	47	18	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	65	40	15	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	70	44	20	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	66	44	20	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	72	42	19	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	59	38	17	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue
	72	42	15	open	thick	pale white/gray	firm	none	none	dead; sediment on tissue

Appendix B Photographs

Photo No.:	**1**
Test Day:	0
Date:	11/22/10
Description: Test bivalves received from Aquatic Research Organisms	

Photo No.:	**2**
Test Day:	0
Date:	11/22/10
Description: Normal mussel tissue from examination (close-up)	

Photo No.:	**3**
Test Day:	**0**
Date:	11/22/10
Description: Normal oyster tissue from examination (close-up)	

Photo No.:	4
Test Day:	0
Date:	11/22/10
Description: Setting up mussels in mesh bags	

Photo No.:	**5**
Test Day:	0
Date:	11/22/10
Description: Setting up oysters in mesh bags	

Photo No.:	**6**
Test Day:	0
Date:	11/22/10
Description: Oysters compartmentalized in mesh bag	

Photo No.:	**7**
Test Day:	0
Date:	11/22/10
Description: Oysters in cage prior to deployment	

Photo No.:	**8**
Test Day:	22
Date:	12/14/10
Description: Retrieving mussel cage at LPR-CBPS1	

Photo No.:	**9**
Test Day:	22
Date:	12/14/10
Description: Mussels from LPR-CBPS2	

Photo No.:	**10**
Test Day:	22
Date:	12/14/10
Description: Normal mussel tissue from LPR-CBPS2 (close-up)	

Photo No.:	**11**
Test Day:	22
Date:	12/14/10

Description: Normal mussel tissue from LPR-CBPS2 (close-up)

Photo No.:	**12**
Test Day:	45
Date:	1/06/11

Description: Retrieving damaged mussel cage at LPR-CBPS2b (no organisms were damaged)

Photo No.:	**13**
Test Day:	45
Date:	1/06/11
Description: Mussels from LPR-CBPS1	

Photo No.:	**14**
Test Day:	45
Date:	1/06/11
Description: Normal mussel tissue from LPR-CBPS1	

Photo No.:	**15**
Test Day:	45
Date:	1/06/11
Description: Normal mussel tissue from LPR-CBPS1 (close-up)	

Photo No.:	**16**
Test Day:	45
Date:	1/06/11
Description: Oysters from LPR-CBPS1	

Photo No.:	**17**
Test Day:	45
Date:	1/06/11
Description: Normal oyster tissue from LPR-CBPS1	

Photo No.:	**18**
Test Day:	45
Date:	1/06/11
Description: Normal oyster tissue from LPR-CBPS1 (close-up)	

Photo No.:	**19**
Test Day:	45
Date:	1/06/11
Description: Mussels from LPR-CBPS2b	

Photo No.:	**20**
Test Day:	45
Date:	1/06/11
Description: Mussels from LPR-CBPS2b (close-up)	

Photo No.:	**21**
Test Day:	45
Date:	1/06/11
Description: Normal mussel tissue from LPR-CBPS2b	

Photo No.:	**22**
Test Day:	45
Date:	1/06/11
Description: Normal mussel tissue from LPR-CBPS2b (close-up)	

Photo No.:	**23**
Test Day:	45
Date:	1/06/11
Description: Surviving oysters from LPR-CBPS2a	

Photo No.:	**24**
Test Day:	45
Date:	1/06/11
Description: Normal tissue from surviving oysters from LPR-CBPS2a	

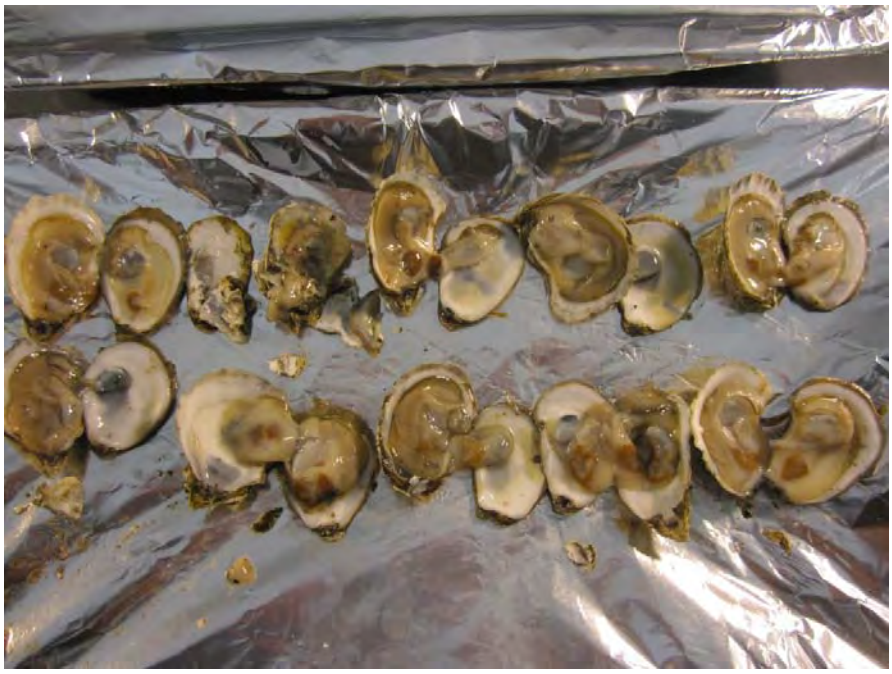


Photo No.:	**25**
Test Day:	45
Date:	1/06/11
Description: Normal tissue from surviving oysters from LPR-CBPS2a (close-up)	

Photo No.:	**26**
Test Day:	45
Date:	1/06/11
Description: Normal tissue from dead oysters from LPR-CBPS2a	

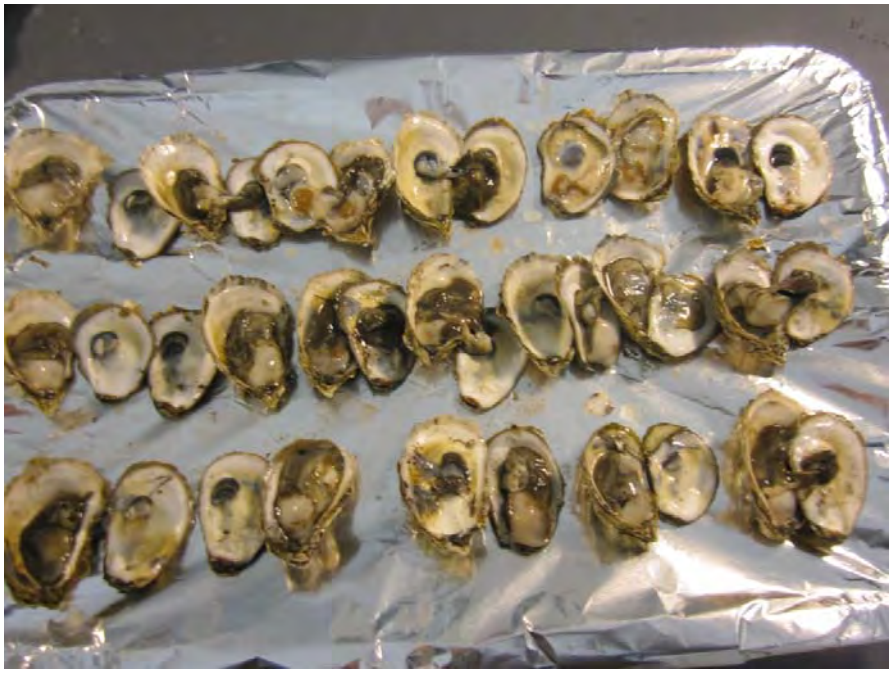
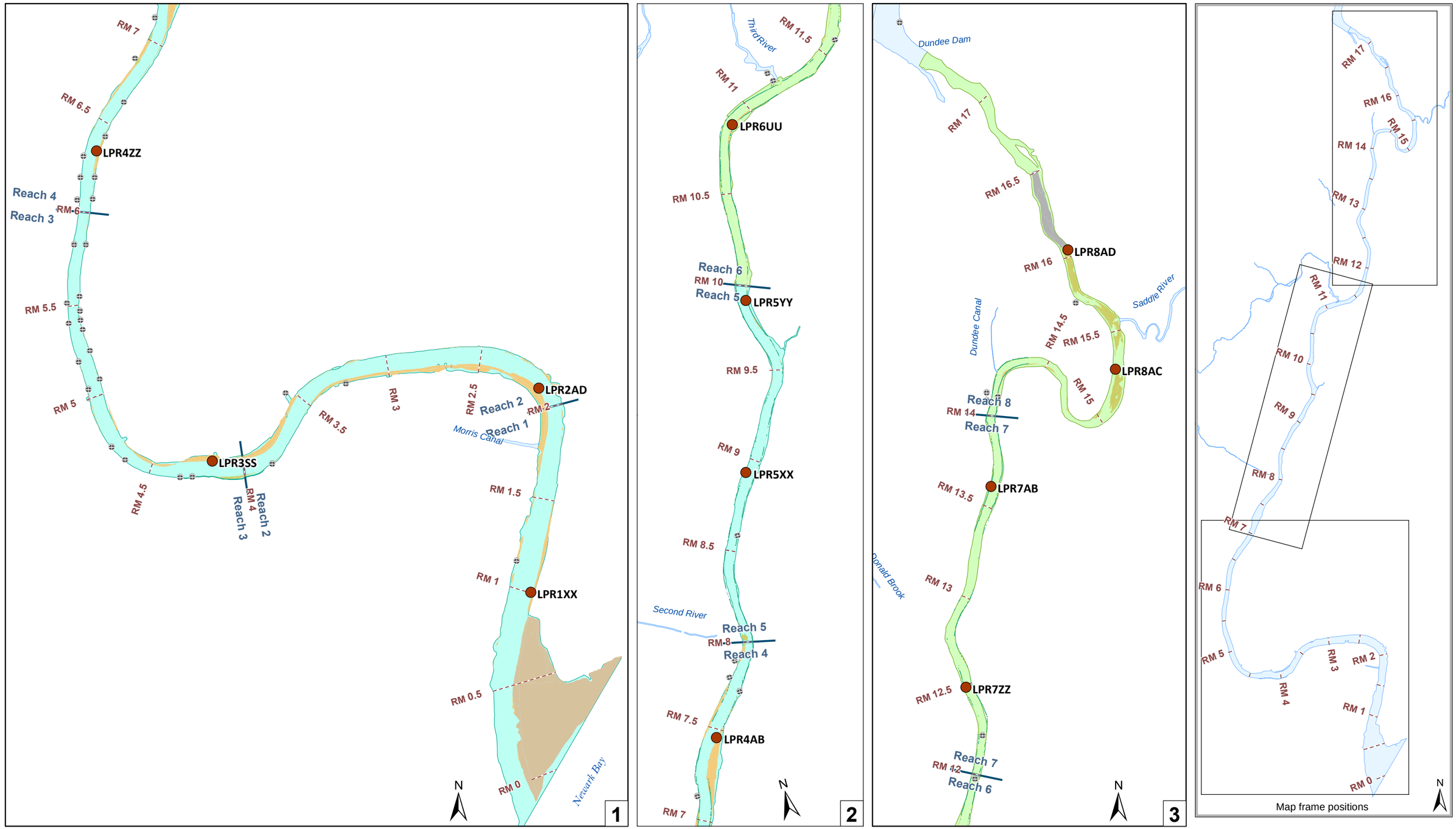


Photo No.:	**27**
Test Day:	45
Date:	1/06/11
Description: Normal tissue from dead oysters from LPR-CBPS2a (close-up)	

Oversize Figures



Wind Ward environmental LLC

0 0.5 1 Kilometers

0 0.5 1 Mile

- Proposed_2011_bivalve_locations
- CSO_locations
- River mile
- Mudflat
- Kearney Point mudflat
- Gravel flat
- No grainsize data
- Sampling reach boundary

LPRSA
- Estuarine river section
- Freshwater river section
- Upper Passaic River or tributary

Mudflat and gravel flat locations are determined as those areas where the river bottom slope is ≤6°and the depth is ≥-4.5 ft NGVD29 (i.e. -2 ft ft MLLW). LPRSA bathymetry is taken from the 2007 bathymetric survey conducted by Gahagan & Bryant Associates, Inc. (GBA), except for the area outside Kearny Point; bathymetry in the southeast part of this area is estimated based on NOAA data. In the GBA survey area, multibeam data are used where available and single-beam data are used where they are not.

Figure 1. Proposed locations for the 2011 LPRSA caged bivalve study

Created by LSM 2/2/11; map 4510; W:\Projects\06-58-01 Passaic RI\Data\GIS\Maps_and_Analysis\Benthic2010